IHC2018-Symposium 2

Micropropagation and In Vitro Techniques (2nd International Symposium)

ORAL PRESENTATIONS

KEYNOTE 1

HOW IN VITRO CULTURE CONTRIBUTE TO PHYTOREMEDIATION

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The environment contamination with different pollutants has become a worldwide problem and the phytoremediation techniques may offer interesting perspectives for counteracting this phenomenon. Many efforts are necessary to improve thesetechniques aimed to the restoration of contaminated soils and waters, and for this purpose, biotechnologies developed in the last decades are now fully available. Phytoremediation takes advantage of differentbiological processes involved in the accumulation, complexation, volatilization, and degradation of organic and inorganic pollutants. In recent years, these processes have been studied on a multitude of species and woody plants, in particular, display the most interesting perspectives for their possibility of accumulating high amounts of specific pollutants in their large biomass. In vitro cultures constitute a powerful tool for developing strategies in phytoremedia tion. Micropropagation and, more in general, cell and tissue culture can be directly exploited in phytoreme diation. For research purposes, these techniques offer the advantage of investigating the plant (or plant cells and organs) behaviour under controlled conditions, in comparison with whole plant experiments under field conditions. Under axenic conditions, we can also distinguish the response of plants from those of microorganisms present in the rhizosphere. The attention must be focused also on the rhizosphere and the complex interactions between plants and microbial communities that may play a relevant role in phytoremediation. These subjects have been largely investigated and a huge literature can be found, so our analysis was mainly addressed towards the field of heavy metals contamination and the use of woody plants for remediation. In the second part, the wide subject of genetic engineering is illustrated; the use of genetically modified plants, in fact, is of great usefulness for the understanding of the metabolic processes involved in the mechanisms of pollutants uptake, sequestration and translocation. Special attention is focused on reactive oxygen species, nitric oxide, and phytohormones, which are signaling molecules modulating plant responses to pollutants stress mainly through differentially expressed genes and the antioxidative system activation. In this context, several genes have been functionally characterized and transformed to target plants for enhancing their phytoremediation efficiency. Consequently, whenever possible, and taking into account any possible risk linked to the use of modified organisms, the use of plants overexpressing genes involved in these phenomena is a promising method for improving the efficacy of phytoremediation.

Keywords: endophytes, heavy metals, micropropagation, mycorrhizas, pollutants

SESSION I: Micropropagation of Fruit Species

OS 1-1:

INCREASED TRANSPIRATION RESULTS IN MORE GROWTH IN IN VITRO GROWN MALUS SHOOTS

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Micropropagation enables vegetative production of large numbers of plantlets in a short period of time. Moreover, it produces in principle vigorous and healthy propagules. The most common method is axillary branching in which multiplication is achieved by forced outgrowth of axillary buds into new shoots. For growth

the conditions in vitro are far from 'normal' and how growth in vitro is achieved is actually still largely unknown. A major question is how medium components are translocated to the areas of growth. Mere diffusion can be ruled out because of Fick's second law of diffusion. We believe that most translocation is through the xylem and uses the flow induced by transpiration. We have shown earlier that in tissue-cultured plants significant transpiration increase occurs(data not shown). It should be noted that reduced transpiration might be one of the reasons of poor performance in tissue culture. In our experiments, shoot cultures of Malus domestica 'Gala' were used to test the relationship of transpiration (stomatal and cuticle transpiration) and growth. The hypothesis is that increased transpiration results in enhanced growth. Apple shoots (Malus x domestica) grown in containers with RH(reduced humidity) showed an increased transpiration and higher fresh and dry weight. Opening the stomata also increased transpiration and biomass accumulation: apple shoots grown in medium with δ-aminolevulinic acid exhibited significantly enhanced stomata aperture and water loss, and showed increase of biomass. We also investigated the effect of the addition of a herbicide that inhibits cuticle formation, metolachlor. The treated plants exhibited increased fresh and dry weight. Our results demonstrated for optimization of biomass accumulation, hence growth, in vitro by increasing transpiration.
Keywords: apple micropropagation, cuticle, stomata, transpiration and growth

OS 1-2:

EFFECT OF SILVER NITRATE ON BACTERIAL CONTAMINATION AND ROOTING OF IN VITRO PLANTLETS OF *Morus nigra* L.

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The effect of silver nitrate on limiting bacterial contamination and improving in vitro rooting were tested on plantlet regeneration of black mulberry. Aseptic culture establishment from field- grown adult woody plants is very difficult due to exogenous and endogenous contamination. Axillary winter buds of a field-grown monumental tree, 100-year-old, were used for shoot proliferation. The excised explants, measuring about 1.0-1.5 cm were thoroughly rinsed with running tap water for 10 minutes, antibacterial soap Protex for 5 minutes, 70% ethanol for 3 minutes, 0.1 % mercuric chloride ($HgCl_2$) for 15 minutes and several times in sterile water to remove the traces of $HgCl_2$. Afterwards the explants were stirred in silver nitrate solution (3 mg/l) for 5 minutes and washed with sterile water. The sterilized buds were cultured on MS (Murashige & Skoog, 1962) medium supplemented with various concentrations and combinations of 6-benzylaminopurine (BAP) (1-1.5-2 mg/l), gibberellic acid (GA_3)(0.2-0.3 mg/l), indole-3-butyric acid (IBA) (0.01 mg/l), polyvinylpyrolidone (PVP) (100-200 mg/l), silver nitrate ($AgNO_3$)(3 mg/l) and sucrose (30%) to induce bud break and shoot multiplication. The high rate bud break and lack of bacterial contamination on establishment stage obtained at 2 mg/l BAP and 3 mg/l $AgNO_3$. For subsequent multiplication and rooting stages the MS medium having $AgNO_3$ and PVP was found suitable to inhibit latent contamination and medium browning. The best multiplication (5-7 number of shoots) was achieved on MS medium containing 1.5 mg/l BAP, 0.2 mg/l GA_3, 0.01 mg/l IBA. Excised in vitro shoots formed roots on the medium containing IBA (1 mg/l) or naphtaleneacetic acid (NAA) (0.5 mg/l). The vigorous plantlet rooting was obtained in ½ MS medium supplemented with IBA (1.0 mg/l). The silver nitrate supplemented culture medium was resulted with highest rooting rate (90%) and the rooted plantlets were hardened in peat + perlite +coconut coir mixture.

Keywords: black mulberry, silver nitrate, micropropagation

OS 1-3:

IN VITRO CULTURE ESTABLISHMENT AND PLANT REGENERATION OF *Arbutus unedo* L.

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Arbutus unedo L. (strawberry tree) grown in around the Mediterranean Region forest area spontaneously. This fruit tree enjoys a growing interest in the world as a result of common uses in the industrial, pharmaceutical and chemical fields. In the present study, the shoot-tip culture and nodal segments of pre-selected three strawberry

tree types were used for in vitro culture. Naturally growing adult-plants were pruned in the autumn. Newly growing shoots (5-10 cm length) were collected to obtain the shoot tip and nodal segments explants for the experiments in the following spring and early summer months. Among the investigated criteria, sterilization procedure, genotype of the donor plants, concentrations of plant growth regulators are evaluated. In the establishment and propagation stages, two basal medium MS and WPM were used. The role of BA alone or with IBA on shoot proliferation, and the effect of IBA on root formation are evaluated.

Keywords: In vitro propagation, regeneration, rooting, browning, strawberry tree

OS 1-4:

STRUCTURAL RELATION FOR ACCLIMATISATION SUCCESS FOR IN VITRO CULTURED AVOCADO

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Sessile nature of land plants has led to structural alterations during evolution as adaptations to dynamic external environments. During in vitro culture plants are grown under most favourable conditions for growth and development and plants are not challenged with changes. Constant temperature, light conditions, water and nutrient supply facilitate the optimum growth but weakens plants ability to survive under external dynamic environment. Acclimatisation is the process of training in vitro plants to natural environment by gradually challenging them with reduced relative humidity, high light intensity, fluctuating temperature and nonsterile environment. Over the last three years Mitter-laboratory at The University of Queensland, Australia has been developing an in vitro mass propagation technology for avocado rootstocks. According to our observations, once rooted some cultivars of avocado have a very high survival at acclimatisation while some cultivars show very poor survival rate. This could be due to structural differences in different cultivars under in vitro conditions. Understanding the in vitro micromorphology and changes occur at different times in the acclimatisation phase will allow customising the acclimatisation process depending on cultivars. The current study will focus on histological comparison of in vitro plants and acclimatised plants of two avocado cultivars; one with high survival at acclimatisation and one with low survival at acclimatisation The changes in foliar epidermal parameters (e.g. stomata, trichomes), venation pattern and root structure during acclimatisation will be investigated. Further, quantitative parameters; trichome density, stomatal density, stomatal index will be compared for the two avocado cultivars to relate the difference in acclimatisation success with plant structure.

Keywords: structure, micropropagation, acclimatisation, avocado

OS 1-5:

STUDY OF OLIVE PLANTS (*Olea Europaea*) OBTAINED WITH MICROPROPAGATION AND CUTTINGS

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With the increasing demand for olive trees (Olea europaea) worldwide, there's a need to develop an efficient technique for large scale production of olive plants with high agronomic and phytosanitary quality. In this study, we compared plants obtained with micropropagation and with cuttings for some agronomic and physiological traits in two olive tree varieties Arbosana and Arbequina. The micropropagation method significantly affected growth, chlorophyll index, chlorophyll fluorescence and stomatal density. The in-vitro plants tended to have a higher stem elongation while the plants from cuttings developed more ramifications that limited the stem growth. The stomatal density was lower in in-vitro plants while no differences were recorded for stomatal conductance and water potential. In Arbosana variety, the root system and leaf area were significantly higher in in-vitro plants in comparison with plants from cuttings. No differences were noted in Arbequina variety. These results obtained from plants grown in greenhouse conditions suggest that in-vitro plants have higher agronomic and

physiological performances that will be economically advantageous for nurseries and farmers. A deeper study will be necessary in order to evaluate the performance of these plants in field.

Keywords: cuttings, micropropagation, *Olea europaea*, agronomic traits, physiological traits

OS 1-6:

PROPAGATION OF SOME CHERRY GENOTYPES AS A CANDIDATE ROOTSTOCK FOR CHERRIES IN DIFFERENT MEDIA IN IN VITRO

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In this study, reproduction possibilities of candidate for cherry and sour cherry genotypes in vitro were investigated. The study was carried out in the tissue culture laboratory of Aksa Agriculture. The lateral and top buds of annual shoots were used as explants and the explants contained 0.1 mg / l IBA (indole butyric acid) + 0.1 mg / l GA3 (gibberellic acid) + 1.0 mg / l BAP (benzyl amino purine) MS (Murashige and Skoog), WPM (Woody Plant Medium) and QL (Quoirin and Lepoivre) media. During the propagation phase, explants were placed in growth chambers containing 16 hours of light and 8 hours of darkness, 25 ± 1 ° C temperature and 2500 lux illumination. Explants were subcultured for 4 weeks. Infection rates in explants taken in June are lower. In the candidate for cherry and sourcherry genotypes, the MS medium has a higher degree of multiplication rate.

Keywords: cherry, rootstocks, in vitro, replication, MS

OS 1-7:

A RESEARCH ON MICROPROPAGATION OF PIXY (*Prunus institia*) ROOTSTOCK

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In recent years, when planting an orchard clonal rootstocks are preferred due to their abilities to obtain early fructification and maximum yield from unit area. In vitro techniques under the heading of plant biotechnology enable rapid and intensive propagation of these rootstocks in laboratory conditions. In this study, reproduction possibilities of Pixy (Prunus institia) one of the important plum clone rootstocks used in plum and apricot cultivation, was investigated by means of tissue culture technique. Shoot tips were used as explants in the study. MS (Murashige-Skoog) was used as the nutrient medium, 30 g / L sucrose, 7 g / L agar added. The shoot tips were placed in the culture room with temperature and light control after planting in the nutrient medium. Samples were taken subculture once a month. Number of explants, number of leaves and number of sprouting were measured every 20 days. During the shoot multiplication phase, six different MS media were tested. The best shoot proliferation was obtained in media supplemented with 4.4 µM BAP + 0.49 µM IBA + 0.29 µM GA3 + 1mM PG. During the rooting phase, four different MS media were tested. It was determined that the best rooting occurred in the nutrient medium containing 1/2 MS + 2 mg/L NAA.
Keywords: Pixy (*Prunus institia*), plum clone rootstock, micropropagation, in vitro conditions.

OS 1-8:

EFFECT OF SOME MODIFICATIONS IN TISSUE CULTURE MEDIA FOR BANANA COMMERCIAL MULTIPLICATION

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The goal of this study was to find cheaper alternatives for the micropropagation of banana (Musa sp.). Low cost options adopted in this study included lowering MS concentration in media, lowering benzylaminopurine (BAP)

in media, and replacement of distilled water with tap water in media preparation. The effect of 4 different concentrations of BAP (0.6 mgL-1,0.8 mgL-1,1 mgL-1,1.2 mgL-1) on bud initiation and shoot length was investigated. The experiment tested the effect of lowering MS concentration in media with 3 different concentrations (3.0 gL-1, 3.5 gL-1, 4.4 gL-1) in order to reduce the cost of media ingredients. The effect of replacing distilled water with tap water was also studied. The investigated concentrations of BAP has shown that using lower concentrations of BAP (0.6 mgL-1, 0.8 mgL-1) will produce slightly lower number of buds while producing higher shoot length if compared to standard concentrations (1 mgL-1, 1.2 mgL-1). Study shows that using low MS concentration (3 gL-1) provides higher number of buds and shoot length when compared to the standard (4.4 gL-1). Using tap water instead of distilled water was effective in lowering cost of production without compromising the quality of plants. Tested media with lowered ingredients (BAP: 0.6mgL-1, MS: 3.0 gL-1, tap water) was tested against standard media and it showed no difference in number of buds while producing higher shoot length plantlets. Using the tested media will cut the cost of media preparation by nearly 50%.

Keywords: banana, tissue culture, micropropagation, growth hormones, tap water

OS 1-9:

NEW APPROACHES TO MICROPROPAGATION OF RASPBERRY

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The possibility of increasing the output of rooted microplants in varieties of remontant raspberry, such as Herakl, Zhar-Ptitsa and PoklonKazakovu was studied. Micropropagation was carried out on the Murasige-Skoog medium with benzylaminopurine (6-BAP) at a concentration of 1.0 mgL-1. For rooting in the control, indolylbutyric acid (IBA) was used to a concentration of 1.0 mgL-1, in the experimental variant auxin was not added. Preliminary study of the micrografts rooting on the nutrient medium of Murashige-Skog on the background of IBA 0.5-1.0 mgL-1 revealed rhizogenesis in the range of 15-40% in all studied varieties with low quality of the roots. In the passage preceding the rooting, according to the traditional scheme, the concentration of BAP was reduced to 0.05 mgL-1 and elongation was carried out in flasks with a volume of 250 ml to obtain micrografts of 2-3 cm in length. In the developed scheme, a separate stage of elongation was not envisaged. In the experimental variant, we combined the stages of elongation and rooting into one and from micrografts of 0.5-1.0 cm length for 1.5-2 months, we obtained rooted microplants, using also large-size cultivation vessels, namely, flasks with a volume of 250 ml. By the end of the second month of growth, the height of the stem part of the microplants of the experimental variant ranged on average from 3.6 to 7.4 cm, and 70-95% of them formed the root system. As a result, the rooting in the experimental variant was 2-3 times higher than the control, no special transplantation required for elongation, for 1.5-2 months accelerated receipt of rooted, well-developed microplants.

Keywords: raspberry, micropropagation, micrografts, benzylaminopurine, auxin

OS 1-9:

PROPAGATION OF MAHLEB BY TISSUE CULTURE

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The aim of this research was to facilitate propagation of some Turkish mahalep genotypes by in vitro techniques. Shoot tips of annual shoots were used as explants. Murashige Skoog (MS) medium was used as base nutrient with 30 g L^{-1} sucrose, and 7 g L^{-1} agar. pH was adjusted to 5,6. The explants were exposed to 16 h light and 8 h dark period at 24 ± 1°C, temperature in growth chamber. The explants were subcultured every 30 days and shoot length, number of leaves and multiplication ratio were observed every 20 days. In multiplication stages, ten different mediums were tested. The optimum multiplication medium was obtained when used MS media supplemented with 4,4 µM BAP+0,49 µM IBA+0, 29 µM GA3. Shoots rooted in MS medium supplemented with 0,3 mg L^{-1} NAA.

Keywords: mahleb, shoot tip, propagation, plant growth regulator

KEYNOTE 2

COMMERCIAL MICROPROPAGATION OF FRUIT VARIETIES AND ROOTSTOCKS BETWEEN TRADITION AND INNOVATION

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In Europe, the production of high-quality fruit varieties and rootstocks from commercial micropropagation accounts for over 70 million plants per year. Italy, Spain, France and Greece are the main producers. Micropropagation, however, is highly labour oriented and, for this reason, outsourcing of in vitro plant production is shifting today to other European countries, having a lower production cost, such as Check Republic, Romania and Turkey. Even more worrying is the competition that, in a near future, could arrive from micropropagation companies located in emerging non-European countries, such as India, Egypt, Iran and China, all countries having expertise in tissue culture and a very low labour cost which allows very cheap nursery productions. The problem is particularly felt in commercial laboratories producing fruit trees and rootstocks where the margin of profit is generally very limited. Hence, large-scale plant micropropagation requires urgently to cut down the cost of production per plant by applying low-cost tissue culture, adopting practices and optimizing use of equipments and resources to reduce the unit cost of micropropagule. At the same time, a very high level of plant quality should be guaranteed. Furthermore, the development and rapid multiplication of new selected cultivars are required to meet the demand of consumers all year round. The scientific activity carried out in research laboratories and Institutions has recently produced important outcomes, not only in the optimization of micropropagation protocols for many economically-important species, but also in the development of innovative *in vitro* techniques, such as the liquid culture in temporary immersion system and the *ex vitro* rooting and acclimatization of plants. The attention is directed also, among others, towards the modernization of laboratory equipment, the improvement of protocols of economically-important species with low proliferation and/or rooting potential, the efficient conservation of shoot cultures in slow growth storage, aimed to increase the laboratory offer of species and cultivars.

Keywords: fruit trees, micropropagation, rootstocks, tissue culture

SESSION 2: In Vitro Techniques: Liquid Culture & Cryopreservation

OS 2-1:

SETIS, A NOVEL VARIANT WITHIN THE TEMPORARY IMMERSION BIOREACTORS

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The temporary immersion system SETIS™ consists of an upper recipient for in vitro plants and a connected lower recipient for liquid medium. The combination of periodical short immersion with nutrients and regular aeration of the headspace allows fast in vitro growth of a large number of plant species. Benefits and drawbacks of the system will be discussed and points of attention and pitfalls during set-up and maintenance will be shared. To illustrate that temporary immersion system require their own medium optimization, a culture of pear in SETIS™ was compared with a classical culture on semi-solid medium.

Keywords: micropropagation, mass production

OS 2-2:

'ElecTIS', A NEW BIOREACTOR FOR LIQUID CULTURE IN TEMPORARY IMMERSION SYSTEM

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Liquid culture in temporary immersion systems (TIS) is an interesting and promising innovation in micropropagation. Although for more than 30 years researchers have worked on developing effective TIS bioreactors, avoiding the use of gelification agents, practical applications of the tecnnique in commercial laboratories for mass propagation are still quite sporadic, and generally limited to species of easy micropropagation. TIS bioreactors are grouped in two categories, i.e., the ones made with one single container (e.g., RITA, Plantform), the others with double containers (e.g., 'twin flasks', SETISTM). In both the categories, the moving element is the liquid medium, which is forced to move (inside the single container, or between containers) by the pressure produced with a pump, pushing air through a gas filter. The use of forced air into the container requires a cereful and continuous control of the efficiency of gas filters and pipe connections, in order to avoid contaminations. For the above, 'ElecTIS' (patented in Europe by Claudio Depaoli, n. 2617282), is an innovative single-container bioreactor which eliminates the use of forced air into the container. It uses instead a mobile basket inside the main box, containing the plant material and moving up and down to allow the immersion in the liquid medium, located in the basal part of the box. The movement of the basket is controlled by a suction pump, connected to the timer. Preliminary experiments demonstrated that (1) high-quality paulownia shoots were obtained by using an immersion cycle of 16 min every 8 hours, without showing any signs of hyperhydricity, (2) chrysanthemum shoots showed an high Relative Growth Rate (RGR, 10.0), in comparison to control shoots in semi-solid medium (RGR, 6.1), and (3) eucalypthus shoots peformed better in 'ElecTIS' (RGR, 14.0), in comparison to Plantform (RGR, 6.2), with an immersion cycle of 4 min every 6 hours.

Keywords: bioreactors, micropropagation, TIS

OS 2-3:

STUDY ON PLANT REGENERATION AFTER CRYOPRESERVING CASSAVA MERISTEMS BY DROPLET VITRIFICATION

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Cassava is an important root crop cultivated in the tropics and source of starch and animal feed. It is a major staple food in the developing world, providing a basic diet for over half a billion people. Pests, diseases and monocultures pose a major threat to its genetic resources. Through cryopreservation, we can protect the wide variety that exists in this species. The droplet vitrification methods was investigated on cassava meristems and the response of 3 different accessions was compared. Apical and axillar meristems were excised from 4, 8 and 16 weeks old in vitro grown plants. Higher viability, shoot growth and plant regeneration percentages were observed for apical shoot tips compared to axillar shoot tips. Overall viability and shoot growth rates where high (average of 78.3% and 73.56%). Plant regeneration percentages, however, were low and varied considerably for the 3 accessions (between 1.26% and 30.9%; average of 11.7%). In order to increase post-thaw regeneration, three different regeneration media were investigated comparing again apical and axillar meristems. Again, the overall viability and shoot growth were high (respectively 91.1% and 77.1%), while the plant regeneration achieved only 10.7%. When the regeneration of the three different accessions are compared, the M. Bra 856 scores significant the best with a percentage of 24.0%, while the other two accessions CM 3306-4 and CM 507-37 reached only a regeneration percentage of respectively 4.4% and 3.9%. Regeneration frequencies on the three

different culture media show no significant differences. The cyto-histological study shows some damaged meristematic zones after the shoot tips underwent cryopreservation. This could be linked to the observation that despite high viability and even shoot growth frequencies are obtained the formation of rooted shoots is still difficult.

Keywords: cryopreservation, cassava

OS 2-4:

CRYOPRESERVATION OF AVOCADO (*Persea americana* mill.) APICAL SHOOT TIPS USING PRE-CULTURE AND VITRIFICATION TECHNIQUES

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Avocado seeds are highly heterozygous and recalcitrant and therefore not suitable for conservation purposes. Presently, avocado germplasm is stored in field collections, which are constantly exposed to abiotic and biotic stresses. Moreover, the size of gene pool, number of replications and quality of maintenance are largely constrained by the local environmental conditions, space and funding. Cryopreservation offers a necessary, complimentary method that is safe, cost-effective and allows for the long-term storage of Persea spp. Currently there are no cryopreservation protocols for storing in vitro shoot tips of Persea spp, which are clonal to the mother tree and thus offer an advantage over somatic embryos, which are zygotic and highly heterozygous. Although not desirable, somatic embryos still offer usefulness in conserving Persea germplasm, however the efficiency of regeneration and quality of the regenerants is often low and genotype dependant. This project has optimised early stages of an in vitro system for shoot tip preservation of avocado cv 'Reed'. Fresh shoot tips were dissected (2mm in size) from clonally propagated adult material and plated on basal media which included varying concentrations of different antioxidants (Ascorbic acid, PVP, Citric acid and Melatonin) to reduce browning of shoot tips. Once optimal antioxidant treatment was selected shoot tips were then pre-cultured overnight in liquid basal medium containing 0.3M sucrose and antioxidant. After pre-culture, tips were loaded (2M glycerol + 0.4M sucrose) for 20 mins and treated with PVS2 at 0°C for 20 mins -LN. Results were recorded for 1) shoot survival %, 2) regrowth of shoot % and 3) shoot biomass. Preliminary trials are revealing the best options for survival and early results indicate that shoots treated in 100 and 250 mg/L of ascorbic acid reduced browning of avocado shoot tips. Further optimization of pre-treatments and PVS2 time points is on going.

Keywords: avocado, cryopreservation, shoot tip, antioxidants

OS 2-5:

THE EFFECT OF ANTIOXIDANT ON REGENERATION RATE OF CRYO PRESERVED WHITE YAM (*Dioscorea rotundata poir*)

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Cryopreservation is the storage of biological samples at ultra-low temperature. It is limited by oxidative stress thereby leading to damage of plant tissues. This study examined the efficacy of different antioxidants in reducing harmful effect of reactive oxidation species in yam cryopreservation. Twenty accessions of white yam were first subjected to droplet vitrification (DV) cryogenic method and four accessions of yam (TDr1662, TDr2071, TDr1710 andTDr4151) were selected, two from the most cryo-amenable ones and two from those with least response to cryopreservation. Lipoic acid (LPA), Ascorbic acid (ASA) and glutathione (GLU) were used at preculture and recovery culture media during cryogenic process using DV method; at different levels of concentration. The effect of the antioxidants is evaluated through the yam meristems regrowth. Results showed that yam meristems regrowth was significantly influenced by the interaction between the genotype and antioxidants used. When ascorbic acid was used, there was always a ASA dose for each accession that promoted regrowth of yam meristems better than the control treatment without antioxidant. In three out of the four accessions tested, meristems regrowth rate was significantly ($p<0.05$) higher compared to control when 25 mg/l

of ASA was added to the preculture and meristems regeneration culture media. The doses of 0.025 and 0.049 mg/l of GLU significantly ($p<0.05$) improved the average meristems regrowth rate for the accessions TDr1662, TDr1710, and TDr2071 compared to control. For TDr4151, the best regrowth rate was observed at the concentration of 0.074 mg/l of GLU. For LPA treatments on TDr1710 and TDr4151, a favorable effect on the meristems regrowth rate at 0.8 and 1.24 mg/l respectively was observed. Hence, ASA at 25 and 40 mg/l, GLU at 0.049 and 0.074 mg/l were recommended for the improvement of regrowth rate in cryopreserved yam. The study concluded that regrowth rate of white yam accessions was improved when the antioxidants were used.

Keywords: cryopreservation, antioxidants, meristem regrowth, secondary metabolites

OS 2-6:

TECHNICAL IMPROVEMENT OF A NEW BIOREACTOR FOR LARGE SCALE MICROPROPAGATION OF HORTICULTURAL CROPS

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Conventional micropropagation is expensive and labour intensive. Until now few laboratories have been truly profitable. With development of a new biological pathway using liquid culture systems in bioreactors it is now possible that micropropagation become cost-effective. We have developed a commercially attractive bioreactor for large scale in vitro plant production as well as for somatic embryogenesis. The bioreactor has been designed in a way that is easy to handle, it has low weight, it is transparent, it is autoclavable. Nutrient supply and gas exchange can be controlled using air pumps and timers. The units can be placed above each other saving place in the climate chamber. Blueberries as well as many other horticultural plants have been tested resulting in high quality plants, faster growth and better survival after transfer to soil. Somatic embryogenesis in Date palm and Norway spruce were improved using the Plantform bioreactor.

Keywords: micropropagation, *in vitro*, bioreactor, horticulture

KEYNOTE 3

PAINS AND GAİNS OF GAMETIC EMBRYOGENESIS IN ALLIUMS

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The genus Allium holds over 850 species including many economically important crop, medicinal and ornamental species. Alliums are highly heterozygous perennials with large genomes. Polyploidy, aneuploidy, and selfing depression are highly common among the species of this genus. Some Allium species such as onion, shallot, Japanese leek, Chinese chive, common chive, domestic and wild leeks, and several other uncultivated Allium species are responsive to gynogenesis-based haploidization technique with varying success. It is, now, possible to obtain plants with reduced ploidy levels from several diploid and tetraploid Allium species by culturing whole flower bud explants on gynogenesis induction media. However, obtaining doubled haploid (DH) fecund plants is still a quite cumbersome process. Success of gynogenic plant production is influenced by many factors including the genetic background of plant material under study. Emergence of gynogenic plantlets (gynogenic response) from cultured buds may take between two to 12 months depending on Allium species under study. About half of the plantlets are lost at this point since they seize to grow. Growing plants are analyzed to determine their ploidy level. In our studies, the highest frequencies haploid plants are obtained from onion and the highest frequencies of dihaploid plants are produced from leek. All haploid and the majority of dihaploid plants obtained are sterile and cannot produce seeds. These plants have to be converted to DH plants in order to re-establish fecundity and obtain seeds. Induced chromosome doubling treatments in hand require use of highly cytotoxic antimitotic agents and lead to generation of many mixoploid plants. Therefore, there is a need

for an efficient method causing low plant mortality and providing high frequency of plants with doubled chromosome number. Fecund gynogenic onion lines are produced by several research groups. Utilization of DH lines as parents in the production of new F1 hybrids is underway and the future for DH onion looks very bright. Leek breeding programs can also benefit from the haploidization technology. Several gynogenic leek lines that were converted to tetraploid level were fecund and provided high numbers of selfed seeds. Leek plants produced from selfed gynogenic lines show very uniform morphological features. Utilization of gynogenic leek lines as pollen donors in the production of new F1 leek hybrids remains to be tested. In this presentation, I will explain difficulties and benefits of utilization of gynogenesis-based gametic embryogenesis technology in Allium improvement programs.

SESSION 3: Micropropagation Of Medicinal And Endemic Plants

OS 3-1:
CHOSEN MEDIA FOR THE SUCCESSFUL *Cannabis sativa* L. IN VITRO INTRODUCTION AND CULTURE IS VARIETY DEPENDENT

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In vitro plant propagation allows to obtain in several crops a higher reproduction rate than the classic vegetative propagation methods, and also it permits to reduce the surface of the area designated for producing the mother plants and for rooting the cuttings. Also, in vitro rooted clones can be more easily transported and shipped abroad due to its sterility. One of the most limiting factor to tackle successfully the first in vitro introduction of a new variety is the different response to the addition of different sources of basal salts mixtures, vitamins, carbon, plant growth regulators (PGRs), and gelling agents. The traditional and most used medium in plant tissue culture is the well-known Murashige and Skoog (MS) based medium, consisting in a mixture of basal salts and vitamins. In our experiments, media was prepared by using, or not, Gamborg (B5) mixture of vitamins and supplemented with 3% sucrose, 0.8% agar and at pH adjusted to 5.8. Two different combinations of phytohormones were used: Meta-topoline vs. Naphthaleneacetic acid + Indole-3-butyric acid. Multiwell plates were incubated under an 18-hour photoperiod at 25ºC for a week and continually monitored. The aim of the investigation was to find the best media for in vitro introduction and culture of six different varieties of Cannabis sativa L. MS was compared to Formula β, an alternative basal salts mixture that has produced significant better results in previous studies (Casano and Grassi, 2009) on this crop. Axillary shoot tips were harvested from young branches of a donor mother plant. Shoot tips of each variety were plated onto MS basal salts medium, as well as onto a Formula β and onto a modified version of Formula β, defined as A. The percentage of survival and germinated shoot tips was recorded, and results indicated that the success was variety dependent.

Keywords: murashige and skoog, formula β, vitamins, axillary shoot tips, plant growth regulators

OS 3-2:
BIOTECHNOLOGICAL TECHNIQUES IN PROPAGATION OF SOME RARE ENDEMIC SPECIES OF THE CRIMEAN FLORA

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Preservation of the gene pool of regional flora rare species is especially relevant. Particularly, the relict endemic species of the Southern Russia flora have so far remained almost unexplored. These species are rare and endangered elements, however many of them are rich in biologically active substances. In nature, they are represented only by some separate populations which renewal is very slow. Explants were subcultured every 3-4 weeks. Culture vessels containing explants were maintained in climatic chamber at a temperature of 20 – 22°C, a 16-hour photoperiod and light intensity 37.5 µmol $m^{-2}s^{-1}$. Some special features of the development under *in vitro* conditions were revealed in the studied relic endemic species, such as *Heracleum ligusticifolium* M. Bieb. (Apiaceae), *Lagoseris callicephala* Juz., *L. purpurea* L. (Asteraceae), *Lamium glaberrimum* (K. Koch) Taliev (Lamiaceae), *Scrophularia exilis* Popl. (Scrophulariaceae), *Silene jailensis* N.I. Rubtzov (Caryophyllaceae) and others. Morphogenetic capacity of various explants types was studied and methods for micropropagation of relict endemic species *in vitro* (embryoculture, tissue and organ culture) had been developed. The knowledge on the studied species biology, development and morphogenesis features has been significantly expanded and deepened. The main ways of morphogenesis in relict endemic species of the Southern Russia flora cultured *in vitro* were first revealed: direct regeneration via adventitious shoot formation and indirect regeneration via somatic embryogenesis. It was demonstrated that MS medium supplemented with 0.1 mg L^{-1} BAP, 0.1 mg L^{-1} IBA and 0.1 mg L^{-1} GA_3 significantly increased regeneration efficiency in the studied species. Physiological assessment of rare species organs and tissues under *in vitro* culture demonstrated their high adaptive ability.

Keywords: rare species, morphogenesis, plant growth regulators, regeneration, photosynthetic activity, *in vitro*

OS 3-3:

ESTABLISHMENT OF HAIRY ROOT LINES AND ANALYSIS OF SAPONIN PRODUCTION IN THE MEDICINAL PLANT *Talinum paniculatum* (JAVANESE GİNSENG)

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The continuous need for new compounds with important medicinal activities has led to the identification and characterization of various plant-derived natural products. As a part of this program, we study the production of potential pharmaceutical compound from *Talinum paniculatum* or widely known as Javanese ginseng. In this research, we reported the induction of transgenic hairy root lines of *T. paniculatum*, screening and selection for saponin production. Two *Agrobacterium rhizogenes* strains, ATCC 15843 and LBA 9402/12 were used to induce hairy roots following infection of leaf explant from in vitro and ex vitro-grown plants. The effects of bacterial concentration, incubation time, and the presence of acetosyringone were also evaluated on the efficiency for hairy root induction. Both strains were able to induce hairy roots from leaf explants, but LBA-induced hairy roots showed faster growth rate than ATCC-induced strain. Saponin analysis from hairy root showed that this type of culture accumulates higher saponin content compare with non-transgenic roots. Thus, hairy roots offer a good system for the production of saponin in *T. paniculatum*.
Keywords: hairy root, javanese ginseng, saponin, *Talinum paniculatum*,

SESSION 4: Micropropagation & In Vitro Embryogenesis Of Ornamentals

OS 4-1:

IN VITRO REGENERATION OF TUBER TISSUES OF WILD CYCLAMEN SPECIES: EFFECTS OF GENOTYPE AND PLANT GROWTH REGULATORS

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Cyclamen is a genus of 22 species of perennial flowering plants in the family Myrsinaceae. Cyclamen species are native to Europe and the Mediterranean Basin. Ten Cyclamen species, 6 of them are endemic, grow naturally in Turkey. Cyclamens are important as ornamental plant as well as medicinal and aromatic plant. In this study, the effects of different plant growth regulators (PGRs) on the regenerations of two wild cyclamen species, *Cyclamen persicum* and *Cyclamen graecum*, were investigated. The tuber tissues as an explant sources were cultured in both flowering and dormant periods for both species. Explants were cultured on ½ MS medium containing different concentration PGRs; 2,4-dichlorophenoxyacetic acid (2,4-D; 0, 0.5, 1, 1.5 and 2 mg L^{-1}) and 6-(γ,γdimethylallylamino) purine (2IP; 0, 0.1, 0.3, 0.5 and 0.8 mg L^{-1}) for regeneration. The results were evaluated by the infection ratio, callus formation and also organogenic and embryonic structure formation. The differences between the genotypes, species, growing periods, and upper-lower parts of the tuber were determined. During the experiment, embryogenic structures were observed from media with 2 mg L^{-1} 2,4-D + 0.3 mg L-1 2IP. The highest number of shoots (100 % of the explants) was obtained on ½ MS medium containing 1,5 mg L^{-1} 2,4-D and 0,8 mg L^{-1} 2IP from *C. persicum* during flowering period. For *C. graecum*, the highest shoot growth (60%) was observed in the combination of 2 mg L^{-1} 2,4-D and 0,8 mg L^{-1} 2IP and all explants formed root like structures in this combination. There were no statistical differences between the upper and lower parts of the tuber. The responds of the explants were dependent on genotype strictly.

Keywords: clonal propagation, genotype effect, tuber

OS 4-2:

EFFECTS OF DIFFERENT SPECTRAL LIGHTS ON PRIMARY AND SECONDARY SOMATIC EMBRYOGENESIS IN *Dianthus caryophyllus*

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Carnation (*Dianthus caryophyllus*) is one of the most important ornamental plants and cut flowers all over the world. It has been proven that many factors can strongly affect not only somatic embryogenesis production and development but also embryo germination. Lighting conditions can affect induction of somatic embryogenesis. To investigate the effects of different light spectrums on somatic embryogenesis of carnations, leaf explants were cultured in Murashige and Skoog medium supplemented with 8 µM picloram, 3% sucrose and 0.6 % agar and were exposed to different spectral lights including white (400-730nm), blue monochromatic light (450 nm), red monochromatic light (635-665 nm) a combination of red and blue (R:B =70:30) and red and far-red (740nm) with the same PPFD (75 µmol m-2 s-1). In current study, primary and secondary somatic embryogenesis were investigated in carnation following exposure to aforementioned light spectrums. The results showed that different spectral lights significantly affected primary and secondary somatic embryogenesis. Red and blue lights caused increase in the percentage of primary and secondary somatic embryogenesis but red+far-red and white lights led to a decrease in primary somatic embryogenesis.

Keywords: light spectrum, carnation, somatic embryogenesis, tissue culture

OS 4-3:

A STUDY OF THE COMPARATIVE GERMINATION RATES AND SEEDLING DEVELOPMENT FOR *Bletilla striata* AND *Bletilla striata* `SORYU´ ORCHIDS USING IN VITRO AND EX VITRO CULTURES

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In this experiment, the germination rates, leaf sizes and protocorm developments were compared for the seeds of two species of orchids, Bletilla striata and Bletilla striata ‘Soryu’. For in vitro culture, 5 different asymbiotic mediums (MS, Phytamax P6668, Phytamax P6668 with coconut water, P723 seed sowing medium, P723 seed sowing medium with coconut water) were used, and also sphagnum moss was used as an ex vitro culture. The

objective of this experiment was to choose best orchid germinationmedia according to germination parameters. For each of the two orchid species, six replicated, each of 70 seeds, were sown in six different media. The germination parameters were observed at 2nd, 3rd, 5th and 8th weeks. At the end of the experiment, the seeds which were used in in vitro culture showed significantly higher parameters than ex vitro. Additionally, Phytamax P6668 medium stimulated the highest seed development until Week 5. However, after week 5, MS medium stimulated the highest seed development. Also, while Bletilla striata showed a germination and seedling development, Bletilla striata 'Soryu' did not show any germination sign over an 8 week period.

Keywords: *Bletilla striata*, orchid, in vitro, ex vitro, asymbiotic germination

OS 4-4:

IN VITRO PROPAGATION AND PRESERVATION OF CHRYSANTHEMUM CULTIVARS AND HYBRID FORMS

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Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) is one of the most popular flowering ornamental plants cultivated all over the world. The biotechnology approaches actively used for propagation and preservation of valuable ornamental cultivars. For our investigation 0.25-0.3 mm meristems and shoot nodal segments (1.0 cm) were used as primary explants. The ways of *in vitro* morphogenetic capacity realization in chrysanthemum were studied. Explants culturing on MS medium with 0.75-1.0 mg L^{-1} BAP and 0.25 mg L^{-1} NAA resulted in formation of hyperhydrated microshoots and development of non-morphogenic callus at their base. Using of MS medium with 0.4-0.75 mg L^{-1} kinetin, 2.5-5.0 mg L^{-1} adenine sulfate and 20.0 g L^{-1} sucrose promoted the growth of well developed adventitious buds and microshoots. For the long-term *in vitro* preservation of viable chrysanthemum explants, optimal concentrations of CCC (chlorocholine chloride) were found out. The presence of CCC promoted inhibition in the chrysanthemum explants growth, compared with the control. Screening of studied cultivars and forms under the effect of different low positive temperatures and CCC within 10 months made it possible to select ones characterized with the maximum viability. Thus, at CCC concentration 0.2-0.4 g L^{-1} the amount of viable explants in chrysanthemum was 90-100%. Explants transferred to the standard cultivation conditions formed adventive microshoots and developed plantlets. It was noted that after the preservation, propagation rate in the studied chrysanthemum cultivars and hybrid forms increased 2-3 times.

Keywords: *Chrysanthemum* × *morifolium* Ramat., explant, morphogenetic capacity, regeneration, conservation, *in vitro*

OS 4-5:

RESCUE AND EX SITU CONSERVATION OF *Mammillaria hernandezii* Glass et Foster (CACTACEAE) THROUGH IN VITRO REGENERATION AND GREENHOUSE GROWTH ESTABLISMENTH

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Mammillaria hernandezii Glass et Foster is a beautiful mexican cactus. The Mexican government (NOM-059-SEMARNAT-2010) and international institutions (CITES) have classifed wild populations under endanger due to overcollections, serious reproductive problems and degration and disturbance of its natural habitat. To contribute to its rescue and possible plant masive propagation and commercialization, we estudied and established the basis for an efficient micropropagation protocol through activation of the axillary buds and shoot proliferation. The effect of a combination of naphtalenacetic acid (ANA= 1 mgL-1) and 6-benzyl-aminopurine (BAP= 0, 3, 4.5 and 6 mgL-1), were evaluated in the production of shoots during the induction step and the best treatments were re-evaluated during proliferation. For the rhizogenesis, MS medium (1962) at 50%

supplemented with four concentrations (0, 0.6 1.2 and 2.4 mg L-1) of indole butyric acid were evaluated. The data obtained revealed that the combination ANA:BAP produce callogenesis and was able to break the dormancy of the buds at all dosages tested but the best treatment during induction and proliferation was ANA 1:BAP 4.5 mgL-1 with 7.2 and 27.8 shoots, respectively. Rhizogenesis was achieved without supplementation of IBA, but its addition to the medium improved the number and length of rotos. 2.4 mg L-1 was the best concentration producing 6.6 roots of 18.5 mm in lenght.

Keywords: auxins, citokinins, consevation, invitro propagation, organogenesis

OS 4-6:

OBTAINING HAPLOID PLANT VIA ANTHER AND OVULE CULTURE TECHNIQUES IN CYCLAMEN

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In this study, haploidization possibilities via ovule and anther culture were investigated in *C. persicum, C.coum, C. hederifolium, C. pseudibericum, C. cilicium* and Melody F1. Anthers were cultured on B5 medium including 0, 1, 3, 5 µM NAA, charcoal (0.5, 1 mg/l), silver nitrate (AgNO3) after 2 days +4°C cold pretreatment. Callus were observed from the anthers of Melody F1 commercial variety cultured on B5 medium including1 g/l charcoal, 0.5 mg/l charcoal + %0.1 AgNO3 +1 µM NAA, 0.5 mg/l charcoal + %0.1 AgNO3+ 3 µM NAA and 0.5 g/l activated coal + %0.1 AgNO3 + 5 µM NAA. In C. persicum species, embryo and plants derieved from these embryos formed from anther explants were cultured on BS medium containing 5µM NAA. Shoot like formations were formed from anthers cultured on B5 medium including 5 µM NAA in C. hederifolium species. As a result of ovule culture experiments of C. persicum and Melody F1 commercial variety, callus, embryo formations and shoots occured from MS medium supplied with 2 mg/l 2,4-D + 0.8 mg/l 2iP and 2 mg/l 2,4-D + 0.5 mg/l 2iP, respectively. In *C. coum* and *C. pseudibericum* species, only callus formations were formed. Embryos occured from MS medium containing 0.5 mg/l 2,4-D + 0.5 mg/l 2iP, 0.5 mg/l 2,4-D + 0.8 mg/l 2iP, 1 mg/l 2,4-D + 0.3 mg/l 2iP in C. cilicium. Callus, embryos, shoot like formations formed in MS medium containing 1 mg/l 2,4-D + 0.5 mg/l 2iP in C. hederifolium species

Keywords: cyclamen, in vitro, MS, B5, haploidy

SESSION 5: Micropropagation & In Vitro Studies In Vegetables

OS 5-1:

AN EFFECTIVE PROTOCOL ON ARTICHOKE (*Cynara scolymus* L.) IN VITRO ROOTING

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Artichoke, belongs to the family *Asteraceae* (*Compositae*), is cultivated in a very wide area in the world and is regarded as functional food due to the bioactive components. The production of artichoke via tissue culture offers considerable advantages. On the other hand one of the most important problems encountered in artichoke tissue culture studies is *in vitro* rooting. The main purpose of the study is, therefore, to develop an effective protocol on *in vitro* rooting of artichoke. For this purpose, after a successful micropropagation process, involving 3 subculture stages, well-developed plantlets were selected and were rooted in different media compositions as ten different media including control group were used in present study. The differences between the media were provided by adding different growth regulators such as IAA, IBA, GA_3, NAA and activated charcoal (0, 1,0 and

2,0 g L^{-1}). Developments of plantlets were observed and recorded with 15 days intervals. According to the results obtained during the study, the medium containing IAA (10,0 mg L^{-1}) and 1,0 g L^{-1} of activated charcoal gave the best results in terms of rooting after micropropagation.
Keywords: artichoke, *Cynara scolymus* L., *in vitro*, rooting

OS 5-2:
IN VITRO STUDIES IN *Allium sandrasicum*

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Allium sandrasicum L. is an endemic diploid (2n=2x=16) perennial Allium species, which is grown in southwestern part of Turkey. The leaves and pseudo-stems of this wild species is edible and consumed as raw or cooked. A. sandrasicum flowers in the first half of summer and produces many viable seeds. There were a few attempts aiming to develop a cultivation system for A. sandrasicum with little success. We initiated a detailed research work to study possibility of developing in vitro culture systems for this valuable wild Allium species. In order to induce somatic and gametic embryogenesis in in vitro, unopened flower buds were cultured in various tissue culture media. Cultures were initiated using flower buds (several days before anthesis) that were collected from greenhouse-grown A. sandrasicum plants between the months of June and July. Buds with somatic and gynogenic shoots were detected after about three to four months of culture. Our findings suggest that presence of both auxin- and cytokinin- type growth regulators (PGRs) were necessary for the induction of somatic regeneration. Induction of gynogenic plant development did not require PGR in cultures. Regenerants were evaluated for their ploidy levels using a flow cytometry (FCM) protocol. FCM analysis showed that all somatic regenerants were diploid. Majority of the gynogenic plants were haploid. There were also mixoploid and diploid individuals among gynogenic plants. Both somatic and gynogenic plants grew well in culture tubes and developed bulbs in in vitro. Results of this study suggest that A. sandrasicum is responsive to somatic and gynogenic plant production in in vitro.

Keywords: *Allium sandrasicum*, flow cytometry, gynogenic, somatic

OS 5-3:
DE NOVO TRANSCRIPTOME ASSEMBLY AND COMPREHENSIVE EXPRESSION PROFILING REVEALS GENES INVOLVED IN HYPERHYDRICITY OF *Allium sativum* L. (GARLIC) PLANTLET IN VITRO

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Hyperhydricity (HH) is one of the most serious problems in plant tissue culture and its underlying molecular mechanism is largely unknown. The identification of genes involved in hyperhydricity of Allium sativum L. (garlic) is critical for a better understanding of its mechanism. Our former study found that low concentration agar induced hyperhydricity. Illumina sequencing technology was used to catalog global gene expression profiles of plantlets after treated with 0.3% and 0.7% agar at 0h, 4h, 24h and 8d. 101298 unigenes were detected, 45133 unigenes were annotated, and 42339 CDS were mapped to protein database. More than 300 genes related to biosynthesis, transport, and response of phytohormones were regulated during three hyperhydricity stages. Differential expression of 30 genes identified by sequencing during the process of HH under different concentration of 6-benzyladenine, hydrogen peroxide and pH were independently confirmed by quantitative real-time-polymerase chain reaction and proved that HH were highly related to balance of reactive oxygen species and plant hormone. Content of reactive oxygen species (hydrogen peroxide and superoxide radicals) and related hormones (cytokinin and ethylene), activities of antioxidant enzymes (catalase, peroxidase, ascorbate oxidase) show a close relationship with hyperhydricity, too. Besides, aquaporin-related Arabidopsis mutants showed higher hyperhydricity than wild type Arabidopsis. Our results shed light on the transcriptional changes that accompany HH and aid in the preventing and recovering of HH of plantlet in vitro.

Keywords: *Allium sativum* L. (Garlic), plantlets in vitro, hyperhydricity, transcriptome, agar

OS 5-3:

GENETICALLY PURE TURKISH ONION (*Allium cepa* L.) LINES

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Onion (*Allium cepa* L.) is an economically important *Allium* species grown all around the world. It is a highly heterozygous biennial diploid plant (*2n= 2x= 16*). Onion is a difficult crop plant to work with since onion genetics and breeding studies may take many years to complete. Turkey is among the major onion producing countries where many land races and open pollinated (OP) standard varieties are commonly used in onion production. Production of pure lines is a major challenge in onion improvement programs. We are carrying out a gynogenesis-based doubled haploid (DH) strategy to obtain fully homozygous onion lines that can be used as potential parents in the development of new hybrid onion varieties. In this project, a total 10 Turkish OP onion (three standard and seven breeding lines developed from land races) lines were used in a two year gynogenesis induction experiments. A total of 387 gynogenic plantlets were developed from all of the donor onion lines. About one fifth of the plantlets continued to grow in *in vitro*. Dried bulbs were produced from 14 DH gynogenic plants. Thirteen of the bulbs that were re-planted in the greenhouse provided selfed seeds (between 63 and 880). Seedlings of DH lines grew uniformly and did not show any sign of breeding depression. Bulbs of these pure lines were comparable to the bulbs of OP donor lines in size, color and other morphological features. These pure onion lines are currently studied for their potential to be used as parental lines in the production of new hybrid onion lines.

Keywords: *Allium cepa* L., doubled haploid (DH), open pollinated (OP), pure line

OS 5-4:

GAMETIC EMBRYOGENESIS IN POLYPLOID EDIBLE ALLIUM

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In this communication, the findings from in vitro gynogenesis induction studies carried out in several polyploid Allium species by our research group were presented and discussed. The genetic improvement of polyploid Alliums is very slow because of high levels of heterozygosity. Gynogenesis-based gametic embryogenesis technique can be utilized to obtain lines with reduced ploidy levels. We attempted to obtain gynogenic plants from two tetraploid and one hexaploid Alliums. Chinese chive (Allium tuberosum) and leek (A. ampeloprasum) are tetraploids (2n=4x=32) and they are mainly propagated through seeds. Elephant garlic is a hexaploid (2n=6x=48) plant closely related to leek. It does not produce seeds and propagated mainly clonally from its large garlic-like cloves. Gynogensis induction experiments were established by culturing large unopened flower buds collected from all plant materials in various tissue culture media. Gynogenic regenerants started to emerge from cultured buds about four months after culture initiation. Chinese chive buds showed a very high response (~100%) and hundreds of regenerants were obtained. Responses of leek and elephant garlic buds were generally low (~1%). For high gynogenesis response, high sucrose concentration was necessary in all induction media. On the other hand, absence of plant growth regulators in induction media did not lead to a significant difference in gynogenesis responses of these three species. Results obtained so far shows that gynogenesis-based haploidization technique can be utilized in the production of plants with gametic chromosome numbers from all three polyploid Allium species included in this study.

Keywords: Chinese chive, elephant garlic, gynogenic, leek, polyploid

POSTER PRESENTATIONS

S2-P1

GROWTH RESPONSES OF POTATO PLANTLETS CULTURED IN VITRO UNDER DİFFERENT COLORS OF LIGHT-EMİTTİNG DİODES (LEDS)

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This exploration work target was to upgrade the micropropagation of potato cultivars through the utilization of new light sources in the development rooms. The experiment consisted of two potato cultivars (Diamant and agria), and five light sources (white, blue, green and red LEDs; and white fluorescent lights). The explants comprised of nodal segments containing one bud, segregated from plantlets developed in vitro. The experimental design was completely randomized arranged in a 2x5 factorial, with 4 replications. Each experimental unit consisted of a flask with five explants. Three 28-day successive subcultures were done in MS medium and incubated under controlled conditions (temperature = 25+2 ºC; photoperiod = 16 hours; light intensity = 20 µmol m-2 s-1). Toward the finish of every subculture, plantlet length, the bud number per plantlet, internode length, plantlet fresh weight, were assessed. After the third subculture, the concentrations of carotenoids and a- and b-chlorophylls were also determined. Different micropropagation efficiencies were established among two potato cultivars developed in vitro conditions: "Agria" was better than " Diamant " cultivar. Different light sources diversely influenced the potato plantlet development: red and green LEDs were the most and least prescribed for plantlet advancement, in view of the after effects of bud number per plantlet, plantlet length, internode length, plantlet FW and plantlet concentrations of a- and b-chlorophylls and carotenoids.

Keywords: *Solanum tubero*sum L., tissue culture, microtuberization

S2-P2

DEVELOPMENT OF IN VITRO MICROPROPAGATION SYSTEM OF *Paeonia lactiflora* Pall. and *Salvia miltiorrhiza*

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Herbaceous peony (*Paeonia lactiflora* Pall.) and *Salvia miltiorrhiza* Bunge are economically important crops in medicinal industry. Peony is usually propagated by division of tuberous root clumps with dormant vegetative underground buds, however, its propagation rate is too low and ornamental properties of stock plants may decrease after division. Several bioactive components from Salvia miltiorrhiza Bunge have long been used for treating cardiovascular diseases. Therefore, we have tried to find their efficient micropropagation protocol, and studied to investigate the effect of medium composition on in vitro shoot induction and plantlet growth from underground buds of herbaceous peony and from nodal explants of Salvia miltiorrhiza Bunge. New buds of herbaceous peony were taken from the underground section of the root neck in March, and used as explants. They were cultivated in the different medium supplemented with various plant growth regulators and

antioxidants for shoot induction and its successive growth. After two weeks of culture, shoots began to emerge and axillary shoot were also formed. After eight weeks of culture, they were fully elongated in multiplication medium. Most shoots rooted successfully after twelve weeks. 1cm nodal segments excised from adult plants of new cultivar 'Dasan'of Salvia miltiorrhiza Bunge were used as explants, and cultivated in the different medium supplemented with various plant growth regulators. After seven to ten days of culture, shoots began to emerge from axillary buds. During culture period, both plantlets showed significantly different responses to the respective medium with different plant growth regulators. Our studies confirmed that shoot induction and plantlet regeneration of herbaceous peony (*Paeonia lactiflora* Pall.) and Salvia miltiorrhiza Bunge might depend on their in vitro reciprocal reactions to the different medium composition and culture condition, and this culture techniques could be an efficient protocols for micropropagation and in vitro manipulation.

Keywords: medium composition, shoot growth, in vitro culture, medicinal plants

S2-P3

IN-VITRO SEEDLING RAISING AND CORMEL FORMATION IN F1 SEEDS OF GLADIOLUS (*Gladiolus grandiflorus* L.)

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The seed setting analysis was carried out in gladiolus based on 5 x 5 full diallel mating utilizing five varieties viz. Her Majesty, Red Ginger, Souvik Late Variety, Summer Sunshine and Sunset Jubilee. The results of the crossings revealed that the average capsule harvest percentage and number of seeds per capsule were found to be 53.43% and 35.36, respectively, and thereafter average germination of the harvested F1 seeds was only 25.30%. Consequently, at the end of the breeding procedures very limited number of seedlings are produced finally that loses a big effort and time of the breeder to achieve hybrid gladiolus varieties. So, an in vitro protocol was developed to solve this problem to regenerate maximum number of plantlets from F1 seeds followed by in vitro cormels production. The experimental results revealed that MS medium fortified with GA3 5.0 mgL-1 and Kinetin 0.5 mgL-1 was found to be the best medium for maximum germination values like germination per cent (99.80%), germination speed (8.69), germination value (14.50) and seedlings' growth attributes like seedling vigour index (1320.67), seedling length (11.50 cm) etc. of F1 seeds. The maximum survival (97.87%) of the in vitro seedlings were found on hardening medium prepared with vermiculite and sand in 1:1 ratio. Secondly, callus was formed on MS medium supplemented with 3.0 mgL-1 2,4-D from root explants of the in vitro raised seedlings. The inducted callus was subcultured on MS medium supplemented with 4.0 mgL-1 BAP and 0.5 mgL-1 NAA for best shooting followed by subcultured on MS medium fortified with 4.0 mgL-1 NAA with 6% sucrose for maximum number of cornlet production. These in vitro raised seedlings or cormels from F1 seeds can be utilised as planting materials for continuation of further breeding program.

Keywords: in vitro, seedling raising, cormel formation, gladiolus, F1 seeds

S2-P4

TRYPSIN INHIBITORY ACTIVITY IN CALLUS OF *Enterolobium contortisiliqqum* (FABACEAE) CULTURED IN GROWING MEDIUM

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The interest in a more sustainable agriculture has favored the increase of researches with plant substances that present biological activity against pathogens and pests. Currently, studies with Enterolobium contortisiliquum have been carried out aiming to characterize and isolate metabolites with biological activity. In this context, the objective of this study was to optimize the trypsin inhibitor expression in callus of E. contortisiliquum cultivated in growing medium and to evaluate the effect of cotyledon and callus extracts on the inhibition of Spodoptera frugiperda trypsin. After asepsis procedures, E. contortisiliquum cotyledons were inoculated in Murashige and

Skoog (MS) medium supplemented with combinations of dichlorophenoxyacetic acid (2,4-D), picloram, kinetin and 6-benzylaminopurine, arranged in a 24-1 fractional factorial design with center point. The levels used were 0.5 mg L^{-1} as the minimum value, 2 mg L^{-1} as maximum and 1.25 mg L^{-1} as the center point. In all treatments, the MS medium was prepared with addition of 30 g L^{-1} sucrose and solidified with 5.5 g L^{-1} agar, prior to autoclaving that occurred at 121ºC for 20 min. Subsequently, the cultures were maintained in a hermetically closed cabinet, with absence of light, in a growth room at 25±2ºC. After 60 days, the trypsin inhibitory activity was evaluated in the cotyledons (control) and in the callus obtained in each treatment. According to the results, the cotyledon of E. contortisiliquum showed the highest anti-tryptic activity (0.0978 UTI/ mL extract). Among the calli produced, the treatment with addition of 0.5 mg L^{-1} 2,4-D + 2.0 mg L^{-1} picloran + 0.5 mg L^{-1} kinetin + 2.0 mg L^{-1} BAP provided an inhibition of 0.0934 UTI/ mL extract. Due to the presence of very close activity values, it is recommended to use this treatment as a form of callus production with higher production of trypsin inhibitors, since this method of culture in a growing medium can be carried out at any time of the year, eliminating thus the seasonal effect of the use of cotyledons.

Keywords: trypsin inhibitor, growth regulator, secondary metabolites

S2-P5

LIGHTING AND SUCROSE IN PHOTOAUTOTROPHIC AND PHOTOMIXOTROPHIC MICROPROPAGATION OF *Physalis angulate*

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Biotechnological interest in Physalis has increased in recent decades. However, there are still few micropropagation researches. An investigation was conducted to evaluate photoautotrophic and photomixotrophic micropropagation of Physalis angulate, gas exchange, seven types of lighting and five concentrations of sucrose. Lighting treatment consisted of yellow, blue, white, red, green, red + blue LEDs and natural light filtered by screen. The concentrations of sucrose were 0, 7.5, 15, 22.5 and 30. Phytotechnical, anatomical and photopigmentation characteristics were evaluated. These consisted of segment, stem and root length, leaf number and area, chlorophyll a, b and, carotenoid content, adaxial and abaxial epidermis, palisade and spongy parenchyma. Data were compared by Scott-Knott's mean test and principal component analysis in software R. Within lighting treatment, only screen, screen-filtered natural illumination, obtained maximum evaluation in all variables. Within sucrose treatment, 15 sucrose obtained the highest number of means with maximum evaluation. The results showed that screen made viable photoautotrophic micropropagation of P. angulata. Furthermore, photomixotrophic micropropagation with 15 g / L of sucrose provided better results.
Keywords: Applied botany; Ecological anatomy; Small fruit crop; Vegetal tissue culture; Photopigments

S2-P6

MICROPROPAGATION OF 'JONGKOLNEE' WATERLILY (*Nymphaea siamensis*)

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The effects of plant growth regulators, 6-benzylaminopurine (BA) and indole-3-acetic acid (IAA), on tissue culture of *Nymphaea siamensis* were studied. Cleaned turions were cultured in liquid Murashige and Skoog medium (MS) supplemented with 0, 2, 3, 4 and 5 mg/l BA combined with 0 and 2 mg/l IAA, followed by sub-culturing of bud explants. A complete randomized design with 9 treatments and 7 replications was used in this experiment. After 60 days, the results revealed that the shoot number and the shoot height were significantly different among the treatments. The maximum average shoot number of 2.86 shoots was observed from MS with 5 mg/l BA and 2 mg/l IAA, while media without the growth regulators gave the maximum shoot height of 0.76 cm and the maximum average leaf number of 11.57 leaves. However, after 90 days, it was observed that MS media with 4 and 5 mg/l BA yielded the maximum number of shoots which amounted to 3.43 shoots on average. Pseudo-turions were found in every treatment after 90 days of culture and the medium with 4 mg/l BA gave the maximum number of pseudo-turions with an average of 2.43 turions per plant. After being transplanted into the natural environment, all plantlets survived and remained healthy.

Keywords: *Nymphaea siamensis*, *in vitro*, plant growth regulators

S2-P8

INDUCTION OF SOMATIC AND GYNOGENIC REGENERATION IN TUNCELI GARLIC (*Allium tuncelianum*)

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Allium tuncelianum (Tunceli Garlic) is a diploid (*2n= 2x= 16*) endemic edible *Allium* species grown naturally in eastern Turkey, particularly in the Munzur mountains. It is popular among the local people of the region due to its single cloved bulb consumed like garlic. *A. tuncelianum* is not a true garlic and can be propagated through seeds. There is an increase in the popularity of the species as a food and over harvesting of *A. tuncelianum* bulbs from nature leads to genetic erosion. Tissue culture-based biotechnological methods can be utilized in mass propagation and genetic improvement of this valuable *Allium* species. A set of experiments were carried out by culturing about 6000 whole flower bud explants collected from greenhouse-grown plants of *A. tuncelianum* in various media. After two months in culture, flower buds grew about five times of their original size. Overall, somatic and gynogenic regeneration were low. Somatic calli were developed from basal parts of the buds cultured in media containing a cytokinin- or cytokinin and auxin-type plant growth regulator (PGR) combinations. Calli were developed from ~1.5% of the buds. However, only six somatic shoots could be produced. Gynogenic regeneration was observed in media containing a combination of cytokinin and auxin-type PGR combinations. We obtained three gynogenic plantlets from buds cultured MS- and BDS-based media. Our results show that *A. tuncelianum* is a recalcitrant species and new tissue culture strategies have to be developed in order to improve somatic and gynogenic plant regeneration.

Keywords: *Allium tuncelianum*, gynogenesis, recalcitrant, somatic

S2-P9

RESPONSE OF PEAR ROOTSTOCK MICROPROPAGATED IN SOLID MEDIUM AND TEMPORARY IMMERSION BIOREACTORS

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Pear is one of the most important temperate fruit crops. Uruguay produces pear of excellent quality with a variable tonnage through years, still being one of the fruits with an important export value. A series of Old Home x Farmingdale (OHxF) pear rootstocks were introduced into the country with the aim of evaluating new materials and trying to identify materials better adapted to local soil and weather conditions. Among those, OHxF 40 was chosen because of its characteristics of lower vigor as well as resistance to fire blight, crown rot, woolly pear aphids, and pear decline. To reach a reasonable number of plants in the shortest possible time,

different in vitro multiplication systems were evaluated: solid media and temporary immersion bioreactors with two different cultivation media in each system. Automation of micropropagation in bioreactors has been considered as a possible way of reducing propagation cost, but it depends upon better understanding of physiological and biochemical responses of plant to the signals of culture microenvironment and an optimization of specific physical and chemical culture conditions to control the morphogenesis of pear plants in this systems. The conventional media of Murashigue Skoog (MS) was compared with an enriched media in three elements: calcium, magnesium and potasium, where the normal amount of those elements in the MS was increased in 2,5 times. In all of the cases 4,44 µM of benzyladenine was used. Significant differences in plant hight were observed, with higher values achieved in the solid media with conventional composition (MS conventional) compared with the media enriched with salts. The highest multiplication rate (24) was observed in temporary immersion bioreactors with media enriched with salts, while the lowest was in solid media with MS conventional composition (4). In none of the cases vitrification was detected.

Keywords: calcium, potassium, magnesium, vitrification, culture medium

S2-P10

RESPONSE OF ANTIOXIDANT SYSTEM ON EXOGENOUS ABSCISIC ACID AND ITS ALLEVIATE EFFECTS ON HYPERHYDRICITY OF GARLIC PLANTLETS IN VITRO

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To investigate the mechanism of exogenous ABA alleviate the hyperhydricity of garlic plantlet in vitro, the effects of exogenous abscisic acid (ABA), hydrogen peroxide (H_2O_2) and H_2O_2 + ABA on hyperhydricity, accumulation and localization of reactive oxygen species, and response of antioxidant system of plantlet in vitro of garlic variety ‘Ershuizao’ were studied. The results showed H_2O_2 aggravated hyperhydricity, the hyperhydric rate was lowest under ABA treatment, and adding ABA into media with H2O2 alleviated hyperhydricity induced by H_2O_2. O_2.- production rate and H_2O_2 content was lowest under ABA treatment and highest under H_2O_2 treatment. Adding ABA to the medium with H_2O_2 reduced the O_2.- production rate and H_2O_2 content. The activities of catalase (CAT), peroxidase (POD) and ascorbate oxidase (APX) increased at the early stage during 0-8 day of H_2O_2 treatment, but then antioxidant enzyme activities of CAT, APX decreased and that of POD increased slowly during 8-16 day. SOD, CAT, POD, GR and APX activities increased linearly at 0-8 d under treatment of ABA and H_2O_2 + ABA. Activities of SOD, CAT, POD and APX under H_2O_2 + ABA treatment were increased and were significantly higher than those of H_2O_2 treatment during 8-16th day. The contents of ascorbic acid (AsA) and glutathione (GSH) increased at first and decreased later, were highest under ABA treatment and lowest under H_2O_2 treatment. Tissue localization results showed that O2.- and H_2O_2 were mainly produced in base of plantlets and tips of leafs. The accumulation of reactive oxygen species was lowest under ABA treatment. Besides, the content of malondialdehyde and membrane relative permeability under treatment of H_2O_2 + ABA was significant lower than H_2O_2 and control treatments. In summary, exogenous ABA can improve the antioxidant capacity of plantlets in vitro, reduce the accumulation and diffuse of reactive oxygen species, thereby inhibiting the occurrence of hyperhydricity.

Keywords: garlic, plantlet in vitro, hyperhydricity, abscisic acid, reactive oxygen species, antioxidant system

S2-P11

IN VITRO DIRECT AND INDIRECT REGENERATION OF PROMISING LAVANDIN CULTIVARS

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Lavandin (*Lavandula* x *intermedia* Emeric ex Loiseleur, Lamiaceae) is one of economically valuable plant, superior to commercially grown lavender cultivars in yield and essential oil output. Traditional vegetative propagation of lavandin is a very slow and laborious process. Biotechnological methods of essential oil plants propagation and cleaning up are the most promising and they enable the development of the essential oil industry in the South of Russia. Plant samples from cultivars ‘Rabat’, ‘Temp’ and clone ‘Snezhnyi Bars’ were collected

in the open field collection of the Nikita Botanical Gardens. After sterilization, meristems 0.5-0.7 mm size and shoot nodal segments were excised and placed on different culture media with plant growth regulators. Initiation of meristem development was observed after 6-7 days on the modified MS and B5 media. After 21-28 days on the media with 0.5-1.0 mg L^{-1} BAP or 0.5-1.0 mg L^{-1} kinetin, with 0.05-0.1 mg L^{-1} NAA and 0.1-0.25 mg L^{-1} GK_3 direct microshoot regeneration was noted in cultivars 'Rabat', 'Temp' and clone 'Snezhnyi Bars'. Callus was formed at the base of microshoots. Histological analysis of callus demonstrated its ability to regenerate morphogenic structures, adventitious buds and microshoots on MS medium with 1.0 mg L^{-1} BAP + 0.25 mg L^{-1} NAA; 0.75 mg L^{-1} kinetin + 2.5 mg L^{-1} adenine sulfate + 0.25 mg L^{-1} NAA and 0.5 mg L^{-1} TDZ + 0.05 mg L^{-1} NAA + 0.5 mg L^{-1} GK_3. Plant regeneration efficiency increased due to indirect regeneration and spontaneous rooting. Thus, the main ways of morphogenesis in three lavandin cultivars were revealed: direct regeneration via adventitious shoot formation; indirect regeneration via callusogenesis, adventitious buds formation and mass micropropagation.

Keywords: *Lavandula x intermedia*, meristem, adventitious microshoot, callus, organogenesis, histological analysis, regenerant, *in vitro*

S2-P12

MORPHOLOGICAL AND ANATOMICAL FEATURES OF THE REGENERATED PLANTS IN LAVENDER (*Lavandula angustifolia* Mill.) AND LAVANDIN (Lavandula x intermedia Emeric ex Loiseleur) CULTIVARS IN VIVO

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Lavender is used as an essential oil, medicinal, aromatic and ornamental plant. In the Crimea, cultivars of lavender and lavandin are in great demand. To improve the plant material, obtain the highest multiplication rate and preserve valuable genotypes, their explants were introduced to *in vitro* culture. Growth and adaptive capacity of the obtained *in vitro* plantlets were studied *in vivo*. The rooted plants were transferred to a mixture substrate. The plants were cultured in the greenhouse for 5 months. Observations of the growth processes were performed every 10 days. Samples for structural studies of vegetative organs were collected *ex vitro* (on 14 day) and *in vivo* – on 60 and 150 days after rooting. Intensive growth *in vivo* was noted from 20th to 60th days. The height of the plants was 9-24 cm (maximum – 'Belyanka' and 'Rabat'). After 150 days during *in vivo* adaptation, the leaf blades were of the same size and shape as those *ex situ*, with typical features of the cultivar. The leaf thickness was 250-274 μm, the leaves were bifacial, hypostomatic, with differentiated 5-7-layered mesophyll and multiple simple and glandular trichomes (52-90 trichomes/mm^2). Stomata functioning became optimal after 60 days (in cultivar 'Belyanka' after 20-30 days). Palisade index was 0.56-0.64. Essential oil drops were noted in epidermal cells, trichomes, leaf mesophyll and parenchyma bundle sheath cells. In the plants of cultivars 'Belyanka', 'Rabat' and 'Snezhnyi Bars' flowering shoots were formed after 120-140 days under *in vivo* culture. Thus, the presented study revealed lavender and lavandin plants ability to form, under *in vivo* conditions, vegetative and generative organs that were structurally and functionally identical to those *ex situ*. Adaptation capacity depended on the genotype and growing conditions.

Keywords: *Lavandula angustifolia*, *Lavandula* × *intermedia*, *in vitro* plantlets, *ex vitro* and *in vivo* adaptation, morphology, structure.

S2-P13

EFFECTS OF EXPLANTS, MEDIA COMPONENTS, CULTURE CONDITIONS, AND EXOGENOUS ADDITIVES ON HYPERHYDRICITY OF *Allium sativum* L.

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Hyperhydricity is a common physiological disorder during plant in vitro culture and seriously affected regeneration and micropropagation of plants. Garlic is very susceptible to hyperhydricity. However, effects of multiple factors on hyperhydricity of garlic remain unclear. To clear the regularity of occurrence of hyperhydricity and to obtain a high-efficiency regeneration system of garlic, we systematically investigated

effects of explants, media components, culture conditions, and exogenous additives on hyperhydricity. Our results showed that shoots were more easily hyperhydric than plantlets. Shoots induced by inflorescences showed a higher hyperhydric rate and proliferation coefficient than those induced by bulbs. Genotype, physiological age, and explant size affected hyperhydricity of shoots in initial culture, not that of plantlets in subculture. Younger inflorescence and smaller explant were more easily hyperhydric. Dose-dependent manners of cytokinins and gelling agents involved in hyperhydricity were found. Hyperhydricity was aggravated at increased cytokinin concentrations and was alleviated by increased gelling agent and sucrose concentrations, ventilation, and illumination intensity. Media with pH higher than 6.0 and lower than 5.8 resulted in more hyperhydricity. Shoots and plantlets were much more likely to be hyperhydric in MS medium than that in B5 medium. Hyperhydricity was relieved by 50μM salicylic acid, 250μM ascorbic acid, 10μM spermidine, and 50μM hydrogen peroxide, but aggravated by high concentrations of hydrogen peroxideand spermidine. Mannitol had no effect on hyperhydricity, whereas polyethylene glycol 6000 induced it. Positive correlations of shoot proliferation and hyperhydricity were found under different treatments of cytokinins, gelling agents, and explants which included genotype, organ type, physiology age and size. A regeneration system of garlic with high proliferation coefficient and low hyperhydric rate was established based on the results above.

Keywords: garlic, hyperhydricity, plantlets in vitro, multiple factors, shoot proliferation

S2-P14

EFFECT OF CHEMICAL FERTILIZERS MEDIUM ON TISSUE CULTURE OF STRAWBERRY

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The Strawberry is a rich in nutrients fruit of which are useful for health in many kinds, such as vitamins A, C, B_1, B_2, protein, calcium, potassium, copper, iron, especially antioxidants. Strawberries are a very popular fruit because the antioxidants in strawberries are much more than in tomatoes up to 7 times. Presently propagation of strawberries requisite the cultivar derived from tissue culture. Because of diseases, infection from the mother plant was resulting in low productivity and chemicals used as bait in vitro are expensive. The aim of this experiment was to replace chemical fertilizer recipe affecting the growth of the strawberry in in vitro. The experiment was conducted using 5x4 Factorial in CRD by used strawberry cultivar Royal 80. The explants were cultured in media containing 5 types of chemical fertilizer as 21-21-21, 15-30-15, 36-5-5, 15-30-15 and 10-52-17 at concentrations of 0.5, 1.5, 2.5 and 3.5 g L^{-1} compared with MS medium was used as the control in each type of fertilizer. The plantlets were cultured in 0.5 g/l of 21-21-21 showed the highest number of plantlets were 4.30 plantlets/explant and better than MS medium (3.44 plantlets/explant), 15-30-15 and 36-5-5 media (3.48, 3.87 plantlets/explant, respectively).

Keywords: strawberry, antioxidants, chemical fertilizers, plantlet, *in vitro*

S2-P15

DETERMINATION OF OPTIMUM METHOD FOR POLYPLOID CUCUMBER (*Cucumis sativus* L.) PLANT REGENERATION OBTAINED BY USING DIFFERENT EXPLANTS

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Cucumber (*Cucumis sativus* L.) and gherkins are important crops with 80,616,692 tonnes production in the world (FAO 2016). It is a high-market plant, both in terms of human health and in terms of easy consumption of delicious and juicy fruits. In order to keep up with the rapidly changing preferences of the developing world, great steps are taken in plant breeding by using technology and science together. It has been attempted to develop an effective procedure for obtaining polyploid plants from different Çengelköy type cucumber (*Cucumis sativus* L.) explants to use in modern breeding studies and plant biotechnology. In the study, chromosomal doubling was performed by applying 0.1% colchicine in three different explant types such as seeds, lateral shoots and apical shoots. Germination of the seeds were carried out in MS0 medium. But the seeds explant, to apply direct colchicine, were kept in the 0.1% colchicine solution for 24 hours, then transferred to MS medium. Apical shoots and lateral shoots were maintained on solid medium containing MS + BAP (0.00023 gr / l) + 0.1% colchicine for

7 days. After that the plants were transferred to MS + BAP (0,00023 gr/l) for rooting. 60 samples were used for each explant types. However, considering the losses, 32 terminal shoots, 48 lateral shoots, 43 seeds survived. Plodiy level of plants was determined by using stoma-chloroplast counting method. In the study, mainly mixoploid plants were obtained. The maximum 34 mixoploid plant were obtained as a result of seeds colchicine application. . According to all explant usage methods, 29 tetraploid plants were formed in total and it was determined that this number was 23.6% when evaluated proportionally. The most successful results were observed in lateral shoots (41.7%) in terms of tetraploid plants obtained.

Keywords: *Cucumis sativus* L., cucumber, poliploid, tetraploid

S2-P16

STEPS TOWARDS MITIGATING CHALLENGES IN THE MICROPROPAGATION OF TROPICAL AND SUBTROPICAL FRUIT CROPS IN SOUTH AFRICA

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Micropropagation was developed as a tool for the rapid propagation of elite plant material. Subsequently, the last half century has seen a variety of regeneration systems being developed, with application in plant breeding and improvement programmes for a wide range of plant species. Unfortunately, both clonal multiplication and the use of *in vitro* manipulation as tools in breeding and improvement programmes of tropical and subtropical fruit crops, are hampered by a number of challenges. Not only do aspects such as plant physiological status, genotype and environmental factors play a role in successful initiation and subsequent manipulation, but characteristics including (but not limited to) high levels of secondary metabolites (e.g. phenolic compounds), tissue texture, the presence of trichomes, as well as exogenous and endogenous pathogens, all play a role in preventing the successful application of *in vitro* manipulation. Tropical and subtropical crop fruit improvement programmes (citrus, mango, avocado, guava, granadilla, banana, macadamia, pineapple, etc.) of the Agricultural Research Council in South Africa focus on the development of selections and cultivars with improved marketable characteristics, tolerance to pests and/or diseases and resilience to suboptimal cultivation conditions. While extensive research is carried out using conventional breeding and selection methods, *in vitro* manipulations are hampered by the factors listed above. Strategies towards the successful initiation and manipulation of field-grown clonal material include pre-induction fungicide, heat and anti-phenolic treatments, while initiation treatments also include various sterilisation agents. An overview of the successes and challenges of tropical and subtropical fruit crop initiation and micropropagation for each of nine crops is presented.

Keywords: tropical and subtropical crops, tissue culture, overcoming challenges

S2-P17

A SUCCESSFUL MICROPROPAGATION PROTOCOL FOR THREE ARONIA (*Aronia melaocarpa*) CULTIVARS

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Aronia (*Aronia melanocarpa* Elliot, black chokeberry) is an important fruit plant for medical and culinary uses. Its' berries are highly nutritive and rich in vitamins and antioxidants. It is a clonally propagated fruit species but there are very few reports of in vitro-based clonal propagation protocols that can be utilized in multiplication of different Aronia cultivars. We developed a highly efficient micropropagation protocol for three Aronia cultivars (Cvs. Eastland, Viking, and Nero). *In vitro* cultures were initiated using actively growing shoots where they were collected from the mature field-grown plants. The shoots were washed thoroughly under tap water, cut into ~10

cm pieces before sterilization procedure. The shoots were dipped in 70 % ethanol for 30 sec. and sterilized in a sterilization solution for 30 min. Then, the shoots were rinsed three times with sterile double distilled water in a Laminar Flow Hood. In order to initiate *in vitro* cultures, nodal explants that were obtained from surface sterilized shoots were placed in Magenta boxes containing MS1 (MSO) and MS2 (MS supplemented with 0.7 mg/l 6-benzylaminopurine (BAP)) media. After about 10 weeks, shoots developed from these initiation cultures were cut into nodal explants and subcultured in MS2 for further multiplication. For rooting, nodal explants were prepared from *in vitro*-grown shoots and cultured in R1 (WPM supplemented with 1 mg/l indole-3-butyric acid (IBA)) and R2 (½ WPM supplemented with 3 mg/l IBA) media. Majority of the explants produced shoots and roots in rooting media within three months. All three cultivars were successfully propagated with slight differences. Rooted plants were successfully acclimated and transferred to a greenhouse for further growth. One year-old plants grew up to 55 cm in height and developed multiple shoots. The protocol developed in this study can be used for *in vitro* clonal propagation of commercially important Aronia cultivars.

Keywords: acclimatization, aronia, clonal propagation, *in vitro*

S2-P18

EFFICIENT REGENERATION OF IMPORTANT VEGETABLE *Brassica oleracea* var. *acephala* from LEAF EXPLANT AND EVALUATION OF ANTIOXIDANT ACTIVITY IN REGENERATED TISSUES

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To improve the yield of *B. oleracea*, considerable research has been conducted to optimize tissue culture and transformation protocols. Brassica spp. are generally considered to be recalcitrant in tissue culture. Leaf explant from 14 days old seed derived plantlets were excised in sterile conditions and inoculated on Murashige and Skoog (MS) medium supplemented with growth regulators. Among the cytokinin, 6-benzyl amino purine (BAP) and Thidiazuron (TDZ) were used alone in the range of 0.1-2.0 mg□L^{-1} or their combinations with α-Naphtalene acetic acid (NAA) or 2,4-Dichloro-Phenoxy acetic acid (2,4-D). Cultures were maintained for four weeks in the culture room at 25±2 ℃, 70% RH and observed on MS media containing 1.5 mg□ L^{-1} TDZ +0.5mg□ L^{-1} NAA. On sub-culturing of the regenerated callus maximum of 91% shoot formation with the higher number of 11 shoots/callus were observed on MS media containing TDZ (2.0 mg□ L^{-1}) + NAA (0.1 mg□ L^{-1}). However, longer shoots of 2.5 cm length were observed on 2 mg□ L^{-1} TDZ containing MS media. Maximum rooting (78 %) of regenerated shoots was achieved on MS rooting medium, MS+1.0 mg□ L^{-1} NAA. Further, we investigated the antioxidant and antimicrobial potential of regenerated shoots and calli extracts and compared with the seed derived plantlets. This plant regeneration protocol could be suitable for further improvement of the agronomic traits of the kale species and will be helpful to raise transgenic lines.

Keywords: kale, regeneration, pgr, antimicrobial, murashige and skoog

This work was carried out in plant cell culture lab, Department of Biotechnology. The consumables for the work were provided by Dr. Bilal Haider Abbasi and seeds for the specie were retrieved from Pakistan agriculture research center.

S2-P19

EFFECT OF AMMONIUM NITRATE (NH_4NO_3) ON GROWTH OF JERUSALEM ARTICHOKE (*Helianthus tuberosus* L.) IN IN VITRO

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Jerusalem artichoke (*Helianthus tuberosus* L.) belongs to Asteraceae (Compositae) family, consisting of high inulin content and valuable source of fructose for application in pharmaceuticals and chemical industries. However, yield and quality of Jerusalem artichoke have been reduced by Pythium. Free-disease plantlet can be produced by tissue culture technique. According to ammonium nitrate (NH_4NO_3) is play important role on plant growth. Therefore, the aim of this study is to investigate the effect of ammonium nitrate on growth of Jerusalem artichoke in in vitro. Auxiliary meristems of field-grown plant were cultured on MS medium containing NH_4NO_3 at 0, 825, 1,650 and 3,300 mgL^{-1} for 4 weeks. The results showed that shoot length, numbers of node, numbers of leaf, leaf width, leaf length, fresh weight and dry weight were highest on 3,300 mgL-1 NH_4NO_3. On the other hand, the highest of plantlet regeneration and rooting were on 1,650 mgL-1 NH_4NO_3. These indicated that high concentration of ammonium nitrate could promote only vegetative growth.
Keywords: ammonium nitrate, jerusalem artichoke, in vitro

S2-P20

EFFECTS OF MS AND MT MEDIUM ON GROWTH FROM NODE EXPLANTS OF *Citrus grandis* L. Osback cv. Manee-Esan IN IN VITRO

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In vitro propagation of *Citrus grandis* L. Osback cv. Manee-esan, which has a juicy pulp with red flesh and a sour- sweetish taste using nodal segments was selected. Cultured on MS and MT medium supplemented with various concentrations of 6-benzylaminopurine (BA) and concentrations of sucrose. After 4 weeks of being cultured, the results showed that the nodal explants cultured on MT medium was superior to MS medium of response promptness and shooting efficiency. The best of shoots induction were obtained when the nodal explants were cultured on MT medium supplemented with 2 mg L^{-1} BA and 50 gL^{-1} sucrose and 500 mgL^{-1} malt extract has the highest number of shoots (1.33 shoots per explants), shoots length (3.21±2.53 mm. per explants), number of leaves (3.69±4.19 leaves per explants) and leaf length (5.23±5.31 mm. per explants). Which in shoot proliferation during in the axillary bud, therefore, obtain to multiple genetically identical shoots.

Keywords**:** pummelo, propagation, BA, tissue culture, nodal segment

S2-P21

EFFECT OF INDUCTION MEDIA ON CALLUS FORMATION IN UN-POLLINATED OVULE CULTURE OF THREE CULTIVARS MELON

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Production of Inbred line such as double haploid plant which is produced from pollen or ovule culture,is one important tool in crop breeding program. Some factors affecting *in vitro* gynogenesis including cultivars of donor plant and induction medias on callus formation in the un-pollinated ovule culture of melon, were investigated. A 3x3 factorial experiment in completely randomized design (CRD) with 5 replications, two factors including three melon cultivars in Thailand (‘Green Net’, ‘Honeydew’ and ‘Pot Orange’) which were used as donor plants, and three induction medias (M1:Murashige and Skoog medium (MS) with 6-benzylaminopurine (BA) 0.20 mg/l and 1-naphthalene acetic acid (NAA) 0.40 mg/l, M2: MS + BAP 0.30 mg/l+ NAA 0.30 mg/l and M3: MS + BAP 0.40 mg/l+ NAA 0.60 mg/l) was conducted. The un-pollinated ovule from melon flower at one day before flowering were used and transferred to induction media in dark condition for 4 days and theirs explants were cultured at LED on daily 16 h light and 8 h dark, 80 µmole.m-2.s-1 for 6 weeks. Callus formation was measured at after being 6 weeks. The interactions among the two factors studied on were no significant. The callus formation potential of all three melon cultivars, showed significantly. The addition of BA 0.20 mg/L and

NAA 0.40 mg/L into the MS medium could induce the highest percentage of callus formation with an average of 75.54%. The callus formation percentages were achieved in several melon cultivars can be implicated for the efficient production of doubled haploid melon in the future.

Keywords**:** 6-Benzylaminopurine (BA), 1-naphthalene acetic acid (NAA), un-pollinated ovule culture, melon

S2-P22

FİELD PERFORMANCE OF DOUBLED HAPLOID (DH) ONION (*Allium cepa* L.) LINES DEVELOPED FROM TURKISH LANDRACES

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Growth and yield performances of five doubled haploid (DH) onion lines developed from three donor (D) Turkish land races were evaluated under field conditions during spring and summer seasons of 2017. Three of the DH lines (DH1, DH2, and DH3) were developed from a landrace producing red bulbs (D1). One DH line (DH4) was developed from a land race with yellow bulbs (D1) and another DH line (DH5) was obtained from a land race with yellow bulbs (D3). Seeds of DH and donor lines were germinated in the greenhouse by sowing them in plastic trays (104 cavities) filled with a mixture of turf and perlite (2:1). Seedlings of DH lines grew well and no abnormal phenotypes were detected among them. All seedlings were transplanted into the field plots as three replicates in the second half of March and cultivated under standard production procedures. Bulbs were harvested in mid-July and placed in a shading house for drying. Dried bulbs were evaluated for bulb traits (color, shape, weight, and size) about one month after the harvest. DH lines showed excellent uniformity in bulb color and shape while donor lines had bulbs showing some variation. There were significant differences among three DH lines developed from D1. Bulbs obtained from DH3 had smaller weight (~50 g) than the bulbs of D1 (~75 g). On the other hand, bulbs of DH2 and DH3 had larger weight than the bulbs of the donor line (>90 g). The weights of bulbs obtained from DH4 and DH5 were comparable to their donor lines (70-90 g). Results of this experiment showed that majority of DH lines developed in our research program performed well under field conditions and produced bulbs with excellent uniformity. These findings suggest that DH lines can be utilized in the production of new onion varieties.

Keywords: *Allium cepa* L., bulb, doubled haploid (DH), land race

S2-P23

MUTATION BREEDING OF STRAWBERRY FOR TOLERANCE TO WATER STRESS USING ETHYL METHANE SULFONATE

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Chemical and physical mutagenesis has been used to increase genetic variability in many plants. Among chemical mutagens, ethyl methane sulphonate (EMS) is the most commonly mutagen used in plants since it causes a high frequency of nucleotide substitutions detected in different genomes. Although *in vitro* propagation of strawberry is simple and easy, *in vitro* mutagenesis studies are very limited, especially under abiotic stress conditions. Water stress is a serious environmental restriction to strawberry productivity. Hence, the objective of this study was to evaluate their effectiveness in inducing mutations and also with the aim of producing variants tolerant to water stress. In the experiment, the effects of different EMS concentrations (0, 0.2, 0.4, 0.6 and 0.8%) and durations (60, 90 and 120 minutes) under *in vitro* polyethylene glycol 6000 (PEG 6000) induced water stress conditions have been investigated using tissue–cultured–meristems on Osmanli, Camarosa and Festival strawberry cultivars. The results showed that water stress tolerant lines could be obtained from induced variations. Explant survival rates, number of shoots, number of roots, root length, number of leaves, plant health index and plant survival rates decreased when EMS concentrations and durations were increased on tried strawberry cultivars. In Osmanli and Camarosa strawberry cultivars, 30% survival rate were determined in control treatment (3% PEG without EMS), whereas 40% survival rate was indicated in ‘No 1’ treatment (60 min

/ 0.2 % EMS) with 3% PEG6000 medium. In the experiment, the highest shoot number, root and leaf number per explant, plant health index and plant survival rates were determined in 'No 1' treatment (60 min / 0.2 % EMS). Depending on increase in concentrations and durations, abnormalities such as dwarfism, color changes on leaves, and aberrant morphologies were observed in plants. The highest morphological variant ratio was 2.22 % for Osmanli cultivar and 'No 7' treatment (60 min / 0.6% EMS) while the lowest ratio was 1.11% in 'No 1' treatment (60 min / 0.2 % EMS) which has been identified under the PEG-induced *in vitro* condition. This study demonstrates the application of *in vitro* mutagenesis in selection of water stress tolerant lines of strawberry as a convenient, cheap, and rapid technique.
Keywords: in vitro mutagenesis, chemical mutagen, PEG 6000, abiotic stress

S2-P24

LEVEL OF MEDIUM SUCROSE, AIR CO_2, AND LIGHT INTENSITY AFFECT GROWTH AND DEVELOPMENT OF MICROPROPAGATED *Dianthus caryophyllus* 'PURPLE BEAUTY'

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Dianthus caryophyllus (carnation) is an important floricultural crop with great ornamental values. Growth and development of carnation 'Purple Beauty' plantlets at a micropropagation stage 3 were studied under two levels each of medium sucrose concentration (0 and 30 $g \cdot L^{-1}$), photosynthetic photon flux density (50 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD), and CO_2 concentration (350 and 1000 $\mu mol \cdot mol^{-1}$). Shoot tip explants, obtained from in vitro-grown plantlets, were stuck and cultured on the Murashige and Skoog (MS) medium in ventilated containers sealed with high efficiency particulate air (HEPA) filter (Nihon Millipore Ltd., Yonezawa, Japan). All cultures were maintained at 24 °C (day) 22°C (night) temperature, 70 (day)/80 % (night) relative humidity, and a 16 h (day)/8 h (night) photoperiod provided by white light emitting diode (LED). Shoot length, root length, fresh weight, and number of roots increased in plantlets cultured in the medium without sucrose (photoautotrophic phase). Number of roots, and plant fresh and dry weights were significantly enhanced in plantlets cultured under a high light intensity (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) as compared with those under a low light intensity (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD). No significant differences in growth were observed in plantlets as affected by concentration of CO_2 supplemented to the culture room.

Keywords: carnation, micropropagation, light intensity, trophic phase

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S2-P25

EFFECT OF CULTURE MEDIA ON IN VITRO CULTURE OF *Rhynchostylis coelestris* Rchb.f

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Rhynchostylis coelestis Rchb.f. ex A.H. Kent is a native orchid of Thailand that currently need to be conserved since it is vulnerable to extinction.The effect of difference media and organic additives for in vitro propagation of *Rhynchostylis coelestis* Rchb.f. ex A.H. Kent was studied. The appropriate medium for protocorm- like bodies (PLBs) and plantlet culture were ½ VM (VW+MS) and ½ MS supplemented with 50 g L^{-1} potato, 50 g L^{-1} banana , 150 ml L^{-1} coconut water and 2 g L-1 activated charcoal. The fresh weight of PLBs and plantlets significantly different with another 3 treatments; VW medium supplement with 150 ml L^{-1}coconut water, orchid fertilizer 21-21-21 2 g L^{-1} and chemical fertilizer 16-16-16 2 g L^{-1} was substituted for chemicals in culture media . These two culture media increased the weight of PLBs by 28 and 30 times in 8 weeks and increased the weight of plantlets by 7 and 6 times in 12 weeks.

Keywords: *Rhynchostylis coelestis* Rchb.f. ex A.H. Kent, in vitro culture, organic additive

S2-P26

POSSIBILITIES TO INHIBIT EXCESSIVE CALLUS FORMATION AND LEAF BROWNING OF HYBRID WALNUT (*Juglans nigra* × *Juglans regia*) IN SHOOT CULTURE

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In vitro shoot culture was established from a promising hybrid walnut clone (Juglans nigra × Juglans regia) for the forestry to micropropagate. Several month later, after a decontamination method leaf browning and excessive callus formation appeared on the basal part and stem of walnut shoots both on multiplication (DKW) and rooting media. The effect of ascorbic acid in different concentrations and active charcoal added to multiplication medium was examined to avoid these symptoms leading to death of the cultures. In the control group on DKW medium leaves got brown and callus formed on the whole plants. Adding 20mg/L or 40mg/L of steril ascorbic acid to DKW medium after autoclaving lead to have more green shoots in the culture but the huge callus formation was not inhibited. An intermediate culturing phase on medium supplemented with charcoal (1.5g/L) decreased the callus formation and leaf browning of walnut shoot culture considerably.
Keywords: hybrid walnut, micropropagation, excessive callus formation, ascorbic acid, active charcoal

S2-P27

EFFECT OF INDOLE-3-ACETIC ACID (IAA) AND 6-FURFURYLAMINOPURINE (NAA) ON IN VITRO ROOT PRODUCTION AND ALKALOIDS ACCUMULATİON OF *Stemona curtisii* Hook. F

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Effect of indole-3-acetic acid (IAA) and 6-Furfurylaminopurine (NAA) on in vtiro root production and alkaloids (stemocurtisine) accumulation of Stemona curtisii Hook. f. in vitro was investigated. Seedlings were cultured on MS supplemented with 0 0.5 and 1.0 mg/L IAA combination with 0 0.5 and 1.0 mg/L kinetin. Roots were harvested after culturing for 2 months. Roots were dried in hot air oven and extracted with 95% ethanol and stemona alkaloids were analyzed by UV-Vis spectrophotometer. The result found that culturing in 0.1 mg/L NAA + 0.1 Kinetin were promoted root production and alkaloids accumulation in in vitro S.curtisii Hook.f with maximum of root fresh, percentage of dry weight, total alkaloid and stemocurtisine contents at 0.40 g, 18.32 %, 44.768 10^{-3}mg/gDW และ 34.82 x 10^{-6} mg /gDW, respectively.

Keywords: stemona, alkaloids, tissue culture

S2-P28

SCREENING OF META-TOPOLIN EFFECTS ON SEVERAL PLANT SPECIES DURING THE IN VITRO GROWTH AND DEVELOPMENT

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The cytokinins (CK) play an important role in controlling of plant growth and development, and the Meta-topolin (mT), an aromatic natural CK, appear as effective alternative to the normally used CK in micropropagation, as 6-benzylaminopurine, zeatin and kinetin. The aim of this study was to evaluate the effect of mT on the micropropagation of several plant species, with agronomic and environmental values, included in five different botanic families: Lamiaceae (Lavandula stoechas subsp. Luisieri), Fabaceae (Pterospartum tridentatum), Fagaceae (Castanea sativa), Actinidiaceae (Actinidia chinensis) and Ericaceae (Vaccinium corymbosum L. and Arbutus unedo). Nodal explants previously established in vitro, were placed in MS or DKW medium with no plant growth regulator (T0), and three different concentrations of mT were used: 2.1 µM (T1), 4.2 µM (T2) and 8.4 µM (T3). New shoots number, length, multiplication factor, color of leaves and

hyperhydricity level were evaluated in two consecutive cycles (30-45 days each cycle, depending of evaluated species). Based on the biometrics parameters, the mT optimum concentration for L. stoechas subsp. luisieri and P. tridentatum in vitro growth and development was 2.1 µM. For these species, was verified that the increasing of mT concentration contributed for increasing of the hyperhydricity level. For Vaccinium corymbosum and Castanea sativa it was found that 4.2 µM mT was better for this species, with higher concentration leading to hyerhydricity. On the other hand, Arbutus unedo and Actinidia chinensis had a better in vitro growth and development on 8.4 µM mT.

Keywords: micropropagation, cytokinins, hyperhydricity, plant tissue culture, plant growth regulators.

S2-P29

THE APPLICATION OF ADAPTED VACUUM INFILTRATION TECHNIC FOR THE IN VITRO ASEPTIC ESTABLISHMENT OF OLIVE TREE (*Olea europea* L.)

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The aim of this study was to establish an efficient disinfection strategy to eliminate the contaminant agents present in the primary explants, for in vitro aseptic establishment of Olive tree (Olea europea L.). Young nodal segments (2cm), collected from a mother plant maintained in greenhouse, were submitted to two different treatments: (T1) Based on Conventional Technic (Water → Deionized water + Detergent → HgCl2 solution → NaOCl solution → Sterile water) and (T2) Based on Adapted Vacuum Infiltration Technic (Water → NaOCl + HgCL2 solution under vacuum infiltration → Sterile water). The treated explants were cultivated on Rugini Olive Medium for 30 days (25oC, 60µmol.m2.s Light intensity and 12 hours light/12 hours dark photoperiod) before evaluations. The results showed a positive effect of the adapted vacuum infiltration technic (T2) for the aseptic establishment of Olive tree explants, comparing with conventional technic (T1). A percentage of 100% cleaned explants was observed on T2 against 46.6% on T1, which had the contaminations with 81.25% of infection only with bacteria and 18.75% with fungus and bacteria. An average of 1.89 new developed shoots with 0.85 cm of length was observed on the explants submitted to T1 against an average of 2.13 new developed shoots with 1.19 cm length for the ones on T2. On T2, 100% of explants showed callus formation (no negative effect) and 12.5% of the produced explants showed some kind of amorphic development, compared to 30% and 1.79%, respectively, on T1. Based on these results, the adapted use of vacuum infiltration could be an efficient technic strategy to eliminate the contaminant agents present in the primary explants, for in vitro aseptic establishment of Olive tree, as well as in other crops.

Keywords: micropropagation, in vitro contamination; disinfection; in vitro plant propagation, mercuric chloride.

S2-P30

EFFECT OF SALINITY ON GOLDEN BERRY (*Physalis peruviana* L.) SEEDS IN IN VITRO CONDITIONS

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The golden berry (*Physalis peruviana* L.) belongs to Solanaceae family and has valuable compounds, such as vitamins (A, B and C), minerals (phosphorus and iron) and antioxidant (β-carotene) for human health. It is referred by different names, cape gooseberry, aguaymanto, uchuva, physalis, Inca berry, Aztec berry or golden berry, in various places of the world. The origin of the golden berry is Peru and it can grow in climate condition where tomato grows. Abiotic stress factors, such as drought and salinity, which are consequences of the global warming, are expected to increase in the future and likely to bring agricultural problems. High salt concentration has a negative effect on chlorophyll which inhibits photosynthesis and consequently plant can die. Also it is difficult to develop salt tolerant plants with conventional methods. In this sense in vitro techniques provide rapid results in the short term. Considering this fact, golden berry provides an opportunity to study abiotic stress tolerance mechanisms for Solanaceae crops. In this study in vitro salinity of golden berry seeds were studied

within the Murashige and Skoog (MS) nutrient medium supplemented with 3% sucrose and 0.7% agar and different concentrations of NaCl (0; 25; 50; 75; 100; 125; 150 and 200 mM) until plantlet formation. Cultured seeds were incubated at 25±20C in growth chamber. Related growth parameters, such as shoot, leaf and root formation, were measured to evaluate salinity effect. Experimental results revealed that different level of salinity treatments in in vitro culture had notable effect on growth parameters as these parameters decreased significantly by increasing salinity level.
Keywords: golden berry (Physalis peruviana L.), salinity, in vitro

S2-P31

RESPONSES OF CHIVE (*Allium schoenoprasum*) AND JAPANESE LEEK (*A. fustilosum*) TO GYNOGENESIS INDUCTION

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Allium improvement programs can benefit from doubled haploid (DH) technology. Allium species are known to be non-responsive to androgenesis induction. In several Allium species, production of haploid and DH plants can be achieved by culturing ovules, ovaries and whole flower buds in induction cultures. We carried out a detailed project to determine the responses of chive and Japanese leek to a gynogenesis induction procedure optimized for onion (A. cepa). Culturing whole flower buds in the plates containing BDS medium resulted in production of gynogenic in both Allium species. Frequency of gynogenic response of chive was medium (~1.5%). We obtained 22 gynogenic chive plantlets of which 11 continued to grow and become plants. Japanese leek performed poorly and a single deformed gynogenic plantlet was obtained. Flow cytometry analysis showed that all regenerants obtained from gynogenesis study were diploids. Diploid chive regenerants were successfully aclimized and grown in a greenhouse for further evaluation to confirm their gametic origin. The low response of Japanese leek may be due to factors such as medium ingredients and genetic background. New gynogenesis induction experiments aiming to determine the influences of medium composition and genetic background of the Japanese leek genotypes are needed.

Keywords: *Allium fustilosum*, *A. schoenoprasum*, gynogenesis, haploid, doubled haploid

S2-P32

EFFECT OF LIGHT INTENSITY AND SUCROSE IN MEDIUM ON GROWTH AND RECOVERY OF *Dianthus caryophyllus* 'PURPLE BEAUTY' FROM HYPERHYDRICITY

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Micropropagation of carnation (*Dianthus caryophyllus*), a popular ornamental plant, is often hindered by hyperhydricity, a physiological disorder with succulent appearance. The Murashige and Skoog (MS) medium, supplemented with different concentrations of sucrose (0, 1, 2, 3, or 4%, w/v), was dispensed in 500 mL glass jars covered with a rigid lid and a ventilation filter [high efficiency particulate air (HEPA) filter (Nihon Millipore Ltd., Yonezawa, Japan)]. The containers were placed randomly in 4 different light intensity regimes, 50, 150, 250, and 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) with a 16 h (day)/8 h (night) photoperiod provided by white light emitting diodes (LED). After six weeks of culture the growth traits, photosynthetic capacity, and biochemical markers such as activities of antioxidant enzymes, and contents of nutrients were measured. The results showed that the growth and development of plantlets were strongly affected by the presence of sucrose in the medium. The sucrose concentration of 0 or 4% gave negative effects on the plantlets under all light intensities. The plantlets still remained in a hyperhydric stage or turned brown along with the low percentage of recovery. However, the culture medium supplemented with 1% sucrose had significantly increased growth parameters, especially those plantlets under light intensity of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. The longest shoot

length and smallest diameter were recorded for the low light intensity (50 µmol·m^{-2}·s^{-1} PPFD). Overall, the outcome of this study can be utilized for optimizing the medium and culture condition for recovery of hyperhydric shoot of carnation in vitro.

Keywords: carnation, micropropagation, hyperhydricity, light intensity, trophic phase
This study was carried out with a support from the Korea Rural Development Administration (Project No. PJ01090805). Luc The Thi, Ji Eun Park, and Swati Das were supported by a scholarship from the BK21 Plus Program, Ministry of Education, Republic of Korea.

S2-P33

GENETIC FIDELITY AND ANTIOXIDANTS ACTIVITY OF IN VITRO PROPAGATED AND FIELD *Musa spp*. MATERIAL FOR PHARMACEUTICAL USE

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Bananas and plantains (Musa spp.L.) are used both as nutritious foods and source of phytoconstituents (secondary metabolites) by the pharmaceutical industry. In vitro propagation is a biotechnological tool that can help to meet the objective of mass plant material production with significant potential for standard and continuous phytochemicals supply. However, trueness-to-type of the clonal material is one of the most important pre-requisites for the use of in vitro-derived of plant materiel. This work assessed the genetic fidelity among the different source of material, estimated total phenolic content (TPC) and to compared the variation in the secondary metabolites activity of field, in vitro culture and acclimatized materials. Selected Musa spp. accessions were micropropagated on Murashige and Skoog (MS) mineral-based culture medium supplemented with 0.18 mg/L of Indole-acetic acid (IAA) and 4.5 mg/L of Benzyl amino purine (BAP). For the genetic fidelity, genomic DNA was extracted from the different Musa accessions and sent for Diversity Array Technology sequencing (DArTseq). Antioxidant activity was evaluated using Ferric-reducing antioxidant power (FRAP) and by DPPH free radical scavenging method, while the total phenolic content (TPC) of both in vitro-grown and field material was determined using the Folin-Ciocalteu reagent method. The pairwise genetic distance matrix based on identity-by-state (IBS) method among the Musa accessionswere determined using 150.5K SNPs and found that 22 out of the 26 accessions were true-to-type. Sebsequent experiments on secondary metabolites were considered on accessions whose genetic fidelity were confirmed. Considerable variation of total phenolic content and antioxidant activity of the Musa spp. were revealed to be genotype-dependant. Accumulation of the useful secondary metabolites, in terms of antioxidant activity, was higher when extracted from in vitro-derived material.

Keywords: Musa spp., in vitro culture, genetic fidelity, antioxidant, DArTseq

S2-P34

TISSUE CULTURE OF *Cannabis sativa* 'EVALUATING THE EFFECTS OF GENOTYPE AND GROWTH REGULATOR COMBINATIONS ON SHOOT GROWTH FROM MERISTEMS AND PLANTLET PRODUCTION

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Three strains of Cannabis sativa L., namely ‘Jesus OG’ (JOG), ‘Pennywise’ (PWE) and ‘Girl Scout Cookies’ (GSC), were tested on Murashige & Skoog (MS) medium for response to shoot growth from meristems. Apical meristems were dissected from shoot tips, surface-sterlized, and placed on full-strength MS containing Gamborg B5 vitamins, sucrose (20 g/L), activated charcoal (1 g/L) and phytagel (3 g/L) (MS-C) supplemented with different growth regulators. Addition of 1 uM thidiazuron (TDZ) and 0.5 uM napthaleneacetic acid (NAA) gave rise to more shoots (90% frequency) compared to giberellic acid and 6-benzylaminopurine, or TDZ alone. Apical

meristems developed into shoots after 6 weeks at 25 ±2oC and at 102 umoles m-2 s-1 light intensity. These shoots were transferred to the same medium for elongation. Four weeks later, the mean heights of shoots of the three strains were not significantly (P=0.05) different (2.83, 2.38 and 2.12 cm for GSC, PWE and JOG, respectively). JOG produced slightly more axillary buds/shoots at 2.13 than GSC and PWE at 1.36 and 1.45, but this difference was not significant (P=0.05). The mean number of axillary shoots at 0.5 (GSC), 0.4 (PWE) and 0.32 (JOG) was not significantly different. Elongated shoots of GSC were transferred onto rooting medium (MS-C containing indole-3-butyric acid), resulting in 76% rooting. Rooted plantlets were successfully acclimatized in soil at a frequency of around 50%.

Keywords: tissue culture, micropropagation, medical marijuana, meristem tip culture

S2-P35

WHITE CABBAGE MICROSPORE RESPONSE TO LOW TEMPERATURE TREATMENT AS A TRIGGER FOR EMBRYOGENESIS

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Brassica group occupies the third place, as importance in the world, due to their high antioxidant, anticarcinogenic, and cardiovascular protective activities conferred to their content in many phytochemicals such as indole phytoalexins, phenolicsand glucosinolates. In this group are included many important vegetable crops, such as cabbage, cauliflower, broccoli and brussels sprouts. Among them, one of the most popular crop is white cabbage (Brassica oleracea var. capitata, forma alba). In Brassica species the obtaining of doubled haploid plants is a key tool for the production of commercial F1 hybrids. Among the many methods employed for this purpose, the culture of isolated microspores offers the opportunity to generate double haploid embryos starting from single haploid cells, thus assuring the genetic purity of haploid plants obtained. Embryogenesis and plant regeneration, the ultimate goals for microspore culture can be achieved by the appplication of different stress treatments that were proved, by the studies, to potentiate the schift between normal gametic evolution of microspore toward the sporophitic one. Despite the multiple studies accomplished, the response of microspores is still not well elucidates, as there are many factors that are involved in this process. The stresses used to determine the sporophytic development of the microspores can be categorized in many ways, the most important ones being according to the time of application (before or during the culture), or type of stress (cold, heat, starvation), etc. The present study is focused in the determination of cold pre-treatments effect applied to buds for 4 to 7 days prior to isolation. The control variant is represented by microspores isolated from freshly cut buds. The results obtained offers an integrative perspective over the effect of cold pre-treatment over the microspore evolution, providing a strong base for the future cultivation protocols at white cabagge.

Keywords: embryogenesis, stress, pollen, *Brassica*, species

S2-P36

ELICITATION OF SECONDARY METABOLITES IN *Musa spp*. USING PLANT TISSUE CULTURE TECHNIQUES

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Musa spp. (bananas and plantains) are staple food crops for millions of people living in tropical countries. They are economically important due to their good dietary value and wide medicinal applications. Therefore, they could be considered as sources of phytoconstituents. Our previous study has shown that antioxidant activity and total phenolic content of in vitro-grown Musa spp. samples were higher when compared to field materials. The aim of this study was to enhance production of secondary metabolites in Musa spp. through abiotic elicitation during micropropagation and to identify the phenolic compounds promoted by the elicitors Six Musa spp. accessions selected based on their antioxidant activity and total phenolic content (2 accessions with high activity, 2 with moderate and 2 low with activity) were subjected to nutrient and culture conditions stress. Different doses of sugar (30-50 g/L), nitrate source reduction (25, 50, 75, 100%) and temperature (15, 20 and 25 °C) were used

as elicitors. The total phenolic content was determined with Folin–Ciocalteu reagent, while the anti-oxidant activity was evaluated with DPPH method. High performance liquid chromatography was used to identify and quantify the phenolic compounds. The level of secondary metabolites and their antioxidant activity varied according to the elicitor use and the dose in addition to the genotype effect. Increase in sugar concentration in the culture medium increased the growth rate of the accessions, while reduced temperature reduced the growth rate of the accessions. This means that an optimal combination should be found, between the elicitation effect and plantlets growth, for in vitro culture to be a reliable alternative system for sustainable and consistent production of biochemical with anti-oxidant activity.

Keywords: *Musa* spp., micropropagation, elicitation, secondary metabolites

S2-P37

MICROPROPAGATION OF *Rosa damascena* USING DIFFERENT TREATMENTS OF PLANT GROWTH REGULATORS

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Rosa damascena is one of the important medicinal plants of Iran and one of the important species of Rosaceae family. The aim of this study was to investigate the micropropagation of this plant in modified MS (Murashige and Skoog) medium with different concentrations of cytokinin (BAP, KIN) for proliferation and different concentrations of auxin (IBA, NAA) for rooting. Single node were used as an explant. Sterilization of the explants was done with 70% ethanol for 30s and then immersed in laundry bleach (5.25 % Hypochlorite sodium) for 10 min and finally washed three times with distilled water. At the stage of establishment, the explants were placed in culture medium without plant growth regulator and then transferred to proliferation stage after one week. Results revealed that the culture medium containing BAP (1.5 mg.l-1) and KIN (0.5 mg.l-1) produced the highest number of shoots/explant and was selected as the best treatment for proliferation. The regenerated shoots then were sub-cultured to the rooting medium. After four weeks, the modified MS supplemented with 0.2 mg.l-1 IBA was the best rooting treatment. For acclimatization, the rooted plantlets were transplanted into the common growth chamber and then in to the greenhouse successfully.

Keywords: in vitro culture, medicinal plants, Rosa damascena, Rooting.

S2-P38

P-COUMARIC ACID INCREASES LIGNIN CONTENT AND REDUCES HYPERHYDRICITY IN IN-VITRO GROWN ARABIDOPSIS SEEDLINGS

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Hyperhydricity (HH) occurs when plants are faced with accumulation of water and reduction of air in the apoplast of the leaves. One of the characteristics of hyperhydric plant is the reduction of cell wall lignification (hypolignification), but how this is related to the abnormalities is still unclear. Lignin is hydrophobic and we speculate that a reduction in lignin levels leads to more capillary action of the cell wall and consequently to more water in the intercellular spaces. p-coumaric acid is a hydroxy derivative of cinnamic acid and a precursor for lignin and flavonoids in higher plants. In the present work, we evaluated the relative amount of apoplast water and air to the total apoplast volume of Arabidopsis thaliana wild-type (Col-0) leaves. Exogenously applied p-coumaric acid can be channelled into the phenylpropanoid pathway thru action of the enzyme 4-hydroxycinnamoyl-CoA ligase (4CL), ultimately resulting in an increase in the total lignin content. Exogenously applied p-coumaric acid led to increases in apoplastic air and lowering of apoplastic water in seedlings grown on medium solidified with Gelrite. The symptoms of HH are also greatly diminished. These findings corroborate our hypothesis that lignin plays a role in the development of the HH and that an increase of lignin production by

exogenously applying p-coumaric acid can lead to a decrease of water in the apoplast and thus to a reduction in the occurrence of HH.

Keywords: hyperhydricity, *Arabidopsis*, apoplast, lignin, *p*-coumaric acid

S2-P39

GENETIC TRANSFORMATION OF SOUR ORANGE ROOTSTOCK WITH iRNA TARGETING *p23* GENE OF CITRUS TRISTEZA VIRUS

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Citrus fruit is one of the most economically important fruit crops in the world. Sour orange (Citrus aurantium), is the main rootstocks in citrus production areas in Turkey. However, our orange is susceptible to Citrus tristeza virus (CTV), the most important virus disease of citrus. Therefore, development of CTV resistant sour orange rootstocks is one of the main objectives of citrus breeding. Development of resistance to CTV using interfering RNA i (iRNA) was previously reported in transgenic Mexican lime (Citrus aurantifolia). In this study, sour orange was transformed with a iRNA targeting CTV p23 gene for exploring possibilities of development of CTV resistance rootstock with this approach. Firstly p23 gene sequence of nearly 400 CTV isolates obtained from in GenBank databases and compared with each other. Two iRNA construct specific to p23 gene of CTV was designed and synthesized. The construct was amplified by PCR and cloned between CaMV 35S promoter and terminator regions in a vector. The resulting gene construct was then cloned into the T-DNA region of pCAMBIA1304 binary plasmid and transferred to Agrobacterium tumefaciens. Epicotyl segments of sour orange seedlings were transformed with A. tumefaciens carrying pCAMBIA1304 binary plasmid with iRNA construct. Transgenic shoots were regenerated from epicotyl pieces by direct organogenesis and then they were rooted or micro-grafted on a rootstock to obtain transgenic sour orange. A total of 1250 epicotyl segments were genetically transformed and 76 shoots were obtained from the transformed epicotyl segments. However, root induction was not achieved in any of these shoots. Therefore, twenty-five of these shoots were micro-grafted on to in vitro grown sour orange rootstock to obtain whole plants. Genomic DNA was isolated 18-newly generated shoots from micro-grafted and non-grafted shoots. The genomic DNA was analyzed by PCR method using primers specific to the iRNA construct targeting CTV p23 gene. PCR analysis revealed that 4 plants contained the transferred gene construct. The results indicated that iRNA approach could be used for developing CTV resistance in sour orange rootstock.

Keywords: citrus, RNA interferans, tristeza, transgenic, resistance

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S2-P40

MICROPROPAGATION OF SELECTED ALMOND (*Amygdalus communis* L.) GENOTYPES

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In the study, the possibility of micropropagation of 42 selected almond genotypes was investigated. For this purpose, shoot tips taken in April were subjected to surface disinfection and then cultured in the MS medium containing 1 mg/L BAP and 0.1 mg/L IBA. The number of shoots per explant, shoot lengths and vitrification situations were determined in the cultures grown in the climate chamber during 6 subcultures. In this respect, the total number of shoots per explant varied between 1.75 and 3.81 in 42 genotypes. In the study, the number of shoots longer than 1 cm ranged between 0.97 and 2.30 shoots/explant, and the number of shoots smaller than 1 cm ranged from 0.75 to 2.04 shoots/explant. Vitrification, which occurs in significant proportions in some genotypes, has negatively affected the micropropagation possibilities of genotypes. At the end of the shoot propagation stage, the healthy and high quality shoots of 14 genotypes (genotypes of 9, 29, 40, 54, 121, 129, 134, 163, 176, 183, 185, 196, 228 and 241) micropropagated effectively were rooted on the MS medium containing half strength macroelements and 1.0 mg/L IBA. Additionally, 120 mg/L of seguestrene was added to

the rooting MS medium as an iron source. The rooting percentages of 14 genotypes were changed between 3.3% and 37.0%. In the study, the rooting percentages of genotypes 9, 29 and 134 were highter than 25%.

Keywords: propagation, in vitro, rooting, plant growth regulators, vitrification

S2-P41

ENHANCEMENT OF IN VITRO PHOTOSYNTHETIC CAPACITY AND GROWTH OF *Epipactis Veratrifolia* ORCHID

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Epipactis veratrifolia is a terrestrial rhizomic wild orchid belongs to temperate zones. Massive propagation through in vitro micropropagation techniques can be utilized for preservation of this orchid. Previous studies showed liquid culture is resulted in better plantlet growth than the solid cultures in vitro. However,establishment of plants is the main concern for plant culture in liquid mediums. In current study, in vitro growth and establishment of E. veratrifolia plantlets were studied using ‘‘Fast’’ liquid medium in combination with different artificial substrates (perlite, Cocopeat and expanded clay (LECA)) as explant stabilizers and ‘‘Fast’’ solid medium was used as the control. Perlite substrate resulted in improvement of all growth parameters (leaf area, root and shoot dry weight, root and shoot fresh weights, root volume, shoot and root lengths, leaf, shoot and root number) and maximum quantum yield of photosystem II (Fv/Fm), while the lowest values for the growth parameters were recorded for Cocopeat and Expanded clay (LECA). Furthermore, the highest value of Fv/Fm was recorded in explant which was cultured in perlite, while the lowest Fv/Fm was observed in Cocopeat-grown explants. Cocopeat as an explant-stabilizing substrate caused a decrease in medium pH, while, LECA caused an increase in medium pH in comparison with the pH of the medium in the start of the culturing practice. However, in perlite substrate, the medium pH was stabilized during in vitro culture. In conclusion, perlite substrarte can be recommended for liquid culture during in vitro production and growth of Epipactis veratrifolia.

Keywords: artificial substrates, perlite, cocopeat, expanded clay, photosystem II

S2-P42

LIGHT QUALITY IN RUTIN PRODUCTION AND GROWTH OF *Physalis angulata* Linn. SEEDLINGS CULTURED IN VITRO

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Known in Brazil as camapú, *Physalis angulata* Linn. is a plant with great importance in folk medicine for its various therapeutic properties and the production of active compounds. P. angulata seedlings were cultured in vitro under different light qualities (white (control), blue, green, red, and yellow) with an irradiance of 50 m^{-2} s^{-1} and photoperiod of 16 h. After 30 days of culture, the shoot length, number of leaves, fresh and dry matter, and rutin content were evaluated. The content of this flavonoid was determined by High-Performance Liquid Chromatography - Diode Array (HPLC-DAD). The mean shoot length was longer in seedlings cultured under yellow light (22.83 ± 0.65 cm, 1.62-fold), red light (22.58 ± 0.44 cm, 1.6-fold), or green light (20.57 ± 0.72 cm, 1.46-fold) than seedlings exposed to white light (14.13 ± 0.26 cm). There were no differences in the mean number of leaves between seedlings grown under the remaining lights and white light. Fresh (1,152 ± 0.16 g)

and dry weight (0.078 ± 0.01 g) were higher in seedlings grown under white light. However, rutin production was higher under blue light (2.78 ± 0.05 µg g^{-1} by dry weight) and green light (2.40 ± 0.06 µg g^{-1} by dry weight). Therefore, the various light qualities affected the growth of P. angulata seedlings cultured in vitro differently, and the blue and green lights promoted greater accumulation of rutin in this species.

Keywords: tissue culture, Physalis angulata, light quality, rutin

S2-P43

ENCAPSULATION OF IN VITRO-DERIVED PROPAGULES OF TWO GENOTYPES OF *Capparis spinosa* (L.) FROM PANTELLERIA ISLAND

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Encapsulation technology, combining the advantages of zygotic or gamic seed with those of micropropagation, has recently attracted the interest of researchers as a new propagation approach, mainly due to the unsatisfying results of the traditional caper propagation strategies. The encapsulation of uninodal microcuttings (3-4 mm long) from two Sicilian Capparis spinosa (L.) genotypes (Tracino and Scauri) was evaluated, observing the influence of the calcium alginate coating and of three different growth regulators (PGRs): 6-Benzylaminopurine (BAP), Meta-Topolin (MT) and Zeatin (ZEA), on viability, regrowth and conversion of the propagules. Microcuttings, were dissected and placed in different Murashige and Skoog-based artificial endosperms, enriched of MT or ZEA or BAP. The synthetic seeds obtained were sown on a medium with full strength salts concentration, enriched with 0,4 mg/L of NAA and 0,7 mg/L of GA3 . After 60 days, the following parameters were detected: viability, regrowth, number and length of the shoots and roots, conversion. The results confirm that encapsulation did not negatively affect the viability, which showed the highest percentage with BAP (100%) in Tracino and with ZEA (100%) in Scauri. Similar results were obtained in regrowth, with statistically significant differences among the three PGRs tested: Tracino showed the best regrowth on capsuled enriched with BAP (100%), Scauri with ZEA (100%). Also the synseed conversion was greatly affected by the PGR, and it was higher in artificial endosperm added with BAP in Tracino (56,6%) and ZEA in Scauri (23,3%) genotype.

Keywords: caper, micropropagation, synthetic seed

S2-P44

REACTIVE OXYGEN SPECIES (ROS) INVOLVED IN SOMATIC EMBRYOGENESIS INDUCTION

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Development of studies to optimize large-scale propagation techniques is considered to be essential in order to establish an effective clonal propagation method. In this context, the somatic embryogenesis provides advantages when compared to other in vitro propagation techniques including high multiplication rate and elimination of the dependence on specific periods of propagative material availability, which allows the establishment of a desirable period to obtain embryos and the possibility to maintain embryogenic cultures through cryopreservation. Even so, all the factors affecting somatic embryogenesis are not known. Some stress factors in tissue culture such as explant injuries, subcultures, unbalanced mineral composition of culture medium and growth regulators may induce the production of Reactive Oxygen Species (ROS). The increasingly interest on the functional meaning of ROS and the antioxidant response concomitant to the growth, development, and cell differentiation in plants, suggest a link between ROS production and morphogenetic processes of plants. Thus, a certain level of oxidative stress may play a significant role activating specific morphogenetic pathways becoming necessary to promote and form embryogenic cells. Hydrogen peroxide (H2O2) as a composition of reactive oxygen species may be generated naturally in a variety of normal cell types, either constitutively or in response to various stimuli. Recent studies show that H2O2 act as cellular ‘messenger’ capable of inducing gene

expression and protein synthesis, thus promoting somatic embryogenesis in some species. Acknowledgments: FAPEMIG, CNPq and CAPES.

Keywords: hydrogen peroxide, embryogenic cells, clonal propagation

S2-P45

MICROPROPAGATION OF PITAYA (*Hylocereus undatus* sp)

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Pitaya (Hylocereus undatus sp) is a very popular fruit in China. It is recently a new alternative fruit in our country. In this research, the clonal propagation capability of red and white fruit flesh cultivars of pitaya which was difficult to propagate by classical methods was investigated. For this aim, young shoots of 30-60 mm length were used as explant. After the sterilization, the explants were put into MS medium with different hormone concentrations.Depending on the cultivar the best results (2,4-5,60 amount/explant) for the amount and the length of shoots were obtained from the MS medium with 2 mg/l BAP, 1 mg/l kinetin, 0.1 mg/ IBA and 0.1 mg/l GA3. After 3 weeks, 100% of rooting was obtained from the shoots in ½ MS liquid medium with 1 mg/l NAA.

Keywords: dragon fruit, ms medium, clonal propagation, shoot amount and length, rooting

S2-P46

PROTOCORMS ENCAPSULATION OF PHALAENOPSIS HYBRIDS (ORCHIDACEAE), TO SCHEDULE IN VITRO PLANTLET PRODUCTION

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Artificial seed production is an alternative technique for asexual propagation and conservation of plants such as orchids with hard natural seed propagation. This technique also rapidly growing in germplasm conservation and plants gene pool exchanges between countries and in vitro floriculture trades. Artificial seed is the beads formed by encapsulating somatic embryo with coating materials. Quality and durability and survival of artificial seed is very important and depends on the kinds of explant, concentration of encapsulation materials and conditions. In this study the effects of different sodium alginate (3, 4 and 5%) and calcium chloride (75, 100 and 150 mM) concentrations for capsule gel matrix were investigated on conversion capabilities and germination of Phalaenopsis artificial seeds. The ideal beads were obtained through a combination of 4% sodium alginate and 150 mM calcium chloride. Encapsulated protocorms showed the best germination percentage (100%) on MS medium after 3 weeks. Storage following capsules desiccation significantly reduced the percentage viability of seeds and protocorms. Treatment with autoclaved distilled water after desiccation and storing resulted in high percentage viability in protocorms whereas dehydrated protocorms were found to be less tolerant to the storing.

Keywords: artificial seed, in vitro, sodium alginate, calcium chloride, phalaenopsis

S2-P47

THE ESTABLISHMENT OF EFFECTIVE REGENERATION SYSTEM AND IN VITRO CULTURE OF CAMPANULA

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Several species of Campanula genus have been cultivated as ornamental plants, and there is an increasing demand for more attractive and novel characteristics. Optimal regeneration protocol is prerequisite for subsequent successful genetic manipulation and generation of new features. In current study the optimal media composition and explant source for shoot regeneration of three Campanula cultivars, C. portenschlagiana Blue Ocean (BO) and Royal; C. carpatica Improved Blue Uniform (IBU) were determined. The focus of this study was based on avoiding over-supplementation of plant growth regulators (PGRs) especially cytokinins (Thidiazuron, TDZ)). The explants which were examined for shoot regeneration were petioles harvested from two different explant sources; 1) Petioles harvested of three months old tissue-cultured plants, 2) Petioles harvested of etiolated nodal cuts. To find out the best media composition for shoot regeneration, different NAA (0.5, 1.0 and 2.0 mg L^{-1}) and TDZ (1.0, 2.0, 3.0 and 5.0 mg L^{-1}) concentrations were investigated. According to the results, the most favorable explant source for harvesting petioles were etiolated nodal cuts of mature greenhouse plants. The best shoot regeneration obtained from the harvested petioles of nodal cuts in the three cultivars were, 85% in cv. IBU, 65% in cv. BO and 45.8% in cv. Royal.

Keywords: shoot regeneration, explant source, TDZ concentration, thidiazuron, PGRs

S2-P48

EFFECT OF DİFFERENT PLANT GROWTH REGULATORS ON SOMATİC EMBRYOGENESİS OF FOUR BEGONİA CULTİVARS

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Begonias are ornamental plants cultivated for their variety of flowers and foliage; they are multiplied by conventional methods by cuttings or in vitro culture techniques. Our work aims to test the effect of growth regulators on the somatic embryogenesis process in four Begonia cultivars: one B. bowerae (1), one B. semperflorens var. hookeri (2), and two B. rex (3 and 4), using limb and petiole explants. We have used basic MS medium with 21 hormonal combinations of BAP (6-Benzylaminopurine), ANA (Naphthylacetic acid) and 2,4-D (2,4-Dichlorophenoxyacetic acid)at different concentrations. The results start to appear from the 4th week of culture, we noticed the formation of filamentous structures and calluses. Varieties (1)and (4)showed excellent reactivity in petiole and limb respectively with the best rate of 64.6% for limb and 100% for the petiole, compared with the other two cultivars.

Keywords: begonia, in vitro, growth regulators, ANA, BAP, 2,4-D, somatic embryogenesis

S2-P49

ALTERNATIVE SUPPORT MATERIALS TO AGAR IN THE IN VITRO CULTIVATION OF *Mouriri elliptica* (Mart.)

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Alternative supports can be successfully used in place of agar for *in vitro* culture to increase seedling vigor. The objective of the present study was to evaluate the efficiency of alternative support materials compared to agar in the *in vitro* cultivation of *Mouriri elliptica* (Mart.) in the absence or presence of naphthalene acetic acid. The alternative support materials used were medium-grain vermiculite, sugarcane (*Saccharum spp.* L.) bagasse and queen palm fiber [*Syagrus romanzoffiana* (Chamisso) Glassman]. No differences were observed between agar, vermiculite and sugarcane bagasse cultures for most growth characteristics evaluated. Greater numbers of adventitious and secondary roots and greater root length were observed in plantlets grown in the presence of vermiculite and the absence of naphthalene acetic acid. In the agar culture, roots had weak points and poorly differentiated tissues, with parenchymal tissue predominating. The concentration of 2.0 mg L^{-1} naphthalene acetic acid used this study did not stimulate rooting of *M. elliptica* (Mart.) plantlets. It was possible to regenerate plantlets in both support materials used, with vermiculite and sugarcane bagasse representing promising agar substitutes to obtain seedlings with roots.

Keywords: anatomical characteristics, melastomataceae, micropropagation, rooting

S2-P50

EFFECT OF MEDIA, STRESS CONDITIONS AND GENOTYPE ON ANDROGENIC PERFORMANCE OF PEPPER

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This study was conducted with the purpose of investigation of the effects of genotype, nutrient media, stress and incubation treatments on haploid plant development on some three-lobed pepper genotypes. İstek F_1, Köylüm F_1, Üçburun F_2 and Tokat local genotype were used as donor plant. DDV (Dumas de Vaulx et al. ,1981) and MS (Murashige and Skoog, 1962) were used as nutrient media. Furthermore, 0.01 mg L^{-1} Kinetin + 0.01 mg L^{-1} 2,4D + 0.03 mg L^{-1} Vitamin B12 were added to the media. Flower buds were held at 4 ^{o}C for 24 hours. Low-temperature treatment was not applied on flower buds of control plants. After anthers were placed in the nutrient media, they were held at 9 ^{o}C and 35 ^{o}C for 8 hours in the darkness. Afterwards, anthers were held at 25±2 ^{o}C for 16 h day / 8 h night for 4 days and transferred to regeneration media. All genotypes that were used in the experiment gave a response to the anther culture more or less. The highest embryo development was obtained from Tokat genotype. Embryo development rates varied between 0.25% and 31.00% by treatments. While embryo development rate was 3.9% in MS medium, it was 9.33% in DDV medium. Moreover, while embryo development rate was 6.7% on anthers that were exposed to cold pre-treatment, it was 6.4% in the control treatment. Embryo development rate in 9 ^{o}C and 35 ^{o}C incubation treatments were 7.60% and 5.50% respectively.

Keywords: anther culture, genotype, stress, incubation, DDV, MS

S2-P51

MANIPULATION OF ADVENTITIOUS ROOTING IN AVOCADO USING NEW TOOLS

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Avocados (Persea) are becoming a major luxury food crop with global demand driving fruit prices to all-time highs. Propagation of elite genotypes requires clonal propagation, a technique that is low efficiency in Avocado due to strong recalcitrance, with current clonal methods both laborious and time intensive. Adventitious roots are produced from non-root tissues, traditionally root primordia formation is stimulated by high auxin levels. Hard-to-root cultivars of woody trees have been shown to reduce bioactive IAA at a faster rate than easy-to-root cultivars. The inability to maintain a high level of IAA is believed to be a strong indicator of recalcitrance. Jasmonic acid (JA) is a plant hormone associated with wound response and herbivory protection, with recent work also linking it with inhibition of adventitious root initiation (Gutierrez et al., 2012). Gretchen Hagen3 (GH3) genes are involved with both IAA degradation and JA activation, making GH3 genes key targets to investigate for promoting adventitious root formation. In this study, novel methods for manipulating the GH3-control of AR were explored using chemical inhibitors and exogenous RNAi in Arabidopsis and the difficult to root species, avocado. Inhibition of IAA conjugating GH3 genes was achieved through application of a competitive ligand, AIEP, previously only used with Grapevine. Early results show an increase in the formation of adventitious roots in Arabidopsis, as well increased root primordia formation in clonal avocado. Exogenous RNAi was also explored as a tool for supressing JA conjugating GH3 genes to induce AR in difficult to root avocado and Arabidopsis.

Keywords: GH3 inhibition, micropropagation, avocado, arabidopsis, RNAi

S2-P52

EFFECT OF ORYZALIN ON IN VITRO REGENERATION FOR POLYPLOIDY TREATMENT IN SULTANA CV. (*Vitis vinifera* L.)

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Efficiency of in vitro chromosome doubling in order to obtain polyploidy mainly depends on explant, culture media and antimitotic agent in a plant. In this study, polyploidization assays have been carried out to determine in vitro explant viability effect of the antimitotic chemical agent oryzalin in Sultana grape cultivars (*Vitis vinifera* L.). Axillary buds and callus were the test materials for chromosome doubling experiments treated with oryzalin, at three concentrations, for three exposure time (24, 48 and 72 h) on two different media (liquid and solid MS medium). Results showed that after oryzalin treatment, the highest viability was obtained from axillary buds in both medium type. The highest regeneration rates were obtained from 20 µM oryzalin treatment for 36 and 48 hours from axillary buds in the liquid nutrient medium of rates 45,83% and 50% respectively.

Keywords: oryzalin, in vitro, axillary bud, callus, sultana cv.(*Vitis vinifera* L.)

S2-P53

THE INFLUENCE OF DIFFERENT CYTOKININ TYPES AND THEIR CONCENTRATIONS ON THE SHOOT PROLIFERATİON OF İN VİTRO CULTURE OF TWO CULTIVARS ROSES (*Rosa hybrida* L.)

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Rose is one of the most beautiful flowers in the world, which has a commercial and ornamental value. In vitro propagation of rose has a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. The experiment was conducted using two varieties of roses. After sterilization and of the explants and the stage of establishment, the single-node explants of *Rosa hybrida* cv. Avalanche and Samourai were cultured on solid and liquid Murashige and Skoog medium (MS) to investigate the influences of different cytokinin types (BAP, BA and KIN) and their concentrations (0.5, 1 and 1.5 mg/l) on the multiplication of shoots. The cultures were maintained at a growth chamber temperature of 23/25°C (night/ day), 80% relative humidity, with a 16-hour photoperiod. The results showed that the highest multiplication rate was obtained in the solid MS medium supplemented with different Concentrations of BAP in both varieties.

Keywords: cytokinin, proliferation, liquid medium, Rosa hybrid.

S2-P54

CRYOPRESERVATION OF TWO WILD SPECIES *Tulipa agenesis* & *Narcissus tazetta* USING V-CRYOPLATE TECHNIQUE

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A new cryopreservation method using aluminum cryo-plates was developed for two selected wild plants from Jordan. Shoot tips of (*Tulipa agenesis*) and callus segments of *Narcissus tazetta* were established on in vitro MS medium. Different treatments were used while applying cryopreservation including different types of loading and PVS (Plant Vitrification Solution) solutions. Furthermore, other treatmentsusing different incubation durations in PVS or cold treatments before cryopreservation on 5 º C for 1 or 3 days were applied. The results revealed that regrowth (66.6%)and survival (100%) of *Tulipa agenesis* shoot tips were maximized when shoottips were dipped in PVS2 solution for 20 min. While for *Narcissus tazetta* calli thehighest regrowth

(83.3%) was obtained when calli segments were dipped in PVS2 solution for 30 min andcomplete survival were obtained at different treatments used. These cryopreservation protocols indicate that we can preserve these two valuable wild species safely using v-cryoplate technique
Keywords: cryopreservation, regrowth, v-cryoplate technique

S2-P55
SOMATIC EMBRYOGENESIS AND PLANT REGENERATION OF CHRYSANTHEMUM FROM DIFFERENT EXPLANTS TYPES

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In vitro plant regeneration, somatic embriyogenesis and somaclonal variation are important tools for plant breeding. In vitro regeneration protocols are also used and necessery for in vitro mutagenesis studies. Furthermore, regenerants derived from different explants may present somaclonal variation. The aim of this study to optimize the regeneration protocols for somatic embryogenesis or organogenesis in two cultivars of Chrysanthemum 'Pusan' and 'Miral Yellow'. Ray florets, leaves and nodes were used as explants. Ray florets were collected from three stages of flower development. Leaf and node explants were excised from in vitro shoots. Explants were cultured on Murashige and Skoog (MS) medium with 3% sucrose, 0.6% agar supplemented with varied concentrations of Benzylaminopurine (BAP), Naphthaleneacetic acid (NAA), Kinetin (KIN) and 2,4-dichlorophenoxyacetic acid (2,4-D) and their combinations. Shoot initiation percentage, number of shoots per explant, length of shoots, number of leaves per shoot, number of nodes per shoot, percentage of embryogenesis and callus rate were recorded in the study. Embriyogenic and regenerated potentials of both chrysanthemum varieties differed according to explant type and growth regulators.
Keywords: In vitro regeneration, ray floret, BAP, NAA

S2-P56
EFFECT OF PACLOBUTRAZOL AND SALICYLIC ACID ON WALNUT IN VITRO ROOTING

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Although protocols are available for micropropagation of walnuts *Juglans* spp., poor rooting is still one of the main factor limiting their micropropagation. Microcuttings of *J. regia*, cv. Chandler, were transferred to a rooting medium consisting in DKW salts and organics, 4 mg L^{-1} IBA, 30 g L^{-1} sucrose, 6 g L^{-1} agar (Sigma) and supplied, in three different experiments, with a) 69 mg L^{-1} salicylic acid, b) increased $CaCl_2$ content (1.5 x) in respect to DKW salt standard composition and c) 1 mg L^{-1} paclobutrazol in absence of IBA. Microcuttings were maintained for 5 days in darkness and then transferred to a hormone free rooting medium and in the light. Rooting medium was used as control in all the experiments. After 40 days from the beginning of the experiments, percentage of explants producing roots, number of roots produced per plant and root length were recorded. Salicylic acid completely inhibited rooting in respect to the control. Similar rooting percentage (around 50 %) and root number were obtained in cv. Chandler with regular or increased $CaCl_2$ contents. Rooting response was improved till around 80%, in respect to the control, when paclobutrazol was applied. Rooting experiments on other *Juglans* spp putatively suitable as rootstocks are in progress.

Keywords: adventitious rooting, cv. chandler, *Juglans* spp.

S2-P57
PRELIMINARY RESULTS ON MICROPROPAGATION OF *Moringa oleifera* Lam

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Moringa oleifera Lam, belonging to Moringaceae family, is a perennial soft-wooded tree known for its multipurpose uses. All parts of the plant are used, expecially for its nutritional and medicinal properties and industrial purpose. Nowadays, for its fast growth and drought-resistance, the interest to introduce this species into cultivation in south Italy areas is increasing. Since not enough good seeds are easy available as planting materials, also because seeds lose germination capacity during the storage after about one year, micropropagation could be a useful technique to overcome this limit. The aim of this research was to develop of an effective in vitro propagation protocol for M. oleifera plants able to obtain high-quality shoot cultures and to promote a large-scale production of plants. Wild seeds of Moringa oleifera coming from Lybia were peeled and surface sterilized (0.05% Hg2Cl2 for 10 minute). Then they were inoculated on agarized sucrose-free modified Murashige and Skoog (BM). The cotyledonary node and axillary buds were excised from the seedlings and cultured on the same medium supplemented with 0.01, 0.05 and 0.5 mg l-1 6-benzylaminopurine (BAP) and 20 g l-1 sucrose. Shoots cultured on BM supplemented with BAP (0.01 mgl-1) showed the best growth without callus formation that allowed the multiplication of the plantlets mainly by microcuttings. Rooting was achieved on the same medium. The rooted plantlets were transferred to a greenhouse where the survival is almost low (50%). Studies are ongoing to increase the survival rate also through the inoculation of arbuscular mycorrhiza fungi.

Keywords: In vitro culture, seeds, 6-benzylaminopurine, acclimatization, survival

S2-P58

MICROPROPAGATION AND CRYOPRESERVATION IN VITRO OF *Carica papaya* L

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Papaya (*Carica papaya* L.) is an important tree fruit crop, with five main producing countries which are India, Brazil, Nigeria, Indonesia and Mexico. Papaya is usually propagated by seed and depending on the domestication degree, trees develop three different sexual forms: male, female, and hermaphrodite. Farmers have preference for hermaphrodite plants because this type self-pollinate and produce more marketable fruits in terms of size, weight, form and quality. Since 1997, AGROMOD started in Chiapas, Mexico the production of papaya 'Maradol' arriving to a production of more than 50,000 tons per year to supply the Mexican and North America market. AGROMOD and NSIP have established the strategy of research and development for papaya breeding using micropropagation and cryopreservation as important tools, pursuing three specific goals. (1) A successful micropropagation protocol has been developed, starting a pilot plantation with more than 70, 000 clonal plants of elite materials through organogenesis by shoot tips, producing true – to – type plants, 100 % hermaphrodite, virus free and homogeneous. (2) Embryo rescue technique plays a significant role in breeding allowing the regeneration of plants coming from seeds of intergeneric hybrids harvested from 30 to 90 days after-pollination, to produce F1 clones via somatic embryogenesis or in vitro germination for field evaluation of morphology traits, resistance or tolerance to virus and fertility. (3) Finally, shoot tips and embryogenic calli were tested to evaluate their regeneration and normal development in response to different cryopreservation techniques: vitrification and slow freezing methodologies were used for 205 papaya accessions genetically characterized and it's being used for long-term conservation of plant germplasm.

Keywords: *Carica papaya* L., micropropagation, organogenesis, somatic embryogenesis, embryo recue, cryopreservation

S2-P59

PROPAGATION IN VITRO OF GISELA 6 AND PHL-C CHERRY ROOTSTOCKS

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In this study, where in vitro propagation posibilities of Gisela 6 and PHL-C rootstocks were determined. The study was conducted tissue culture loboratory of Blacksea Agriculture Research Institue. Apical buds of annual shoots were used as explant materials. For this purpose. explants were cultured on MS medium suplemented with different plant growth regulator combinations MS (Murashige and Skoog), medium containing 0.5 mg/l BAP (benzil amino pürin) + 0.2 mg/l GA3 (gibberalic acid) + 0.0, 0.01, 0.05 and 0.1 mg/l IBA (indole butyric acid). It was used rooting media ½ MS 0.0, 0.5, 1.0 and 2.0 mg/l IBA. The cultures were incubated in a culture room at 26±2 0C with a 16-h photoperiod under 2500 lux light irradiance. The explants were taken with an interval of 4 weeks subculture and was calculated. The shoot numbers were changed between 2.22 and 4.26 shoots per explant for Gisela 6, between 1.50 and 4.67 shoots per explant for PHL-C, according to treatments. The highest rooting rate was 1 mg/l IBA application in Gisela 6 while PHL-C was obtained from 0.5 mg/l IBA application.

Keywords: Gisela 6, PHL-C, in vitro, propagation, rootstocks

S2-P60

SLOW GROWTH IN APRICOT CV. PISANA AND IN APPLE CV. CERINA

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The storage of micropropagated plants in slow growth has the aim to reduce the frequency of periodic sub-culturing. The most commonly applied conditions include low temperature, darkness and addition of sugars to the medium. Encapsulation technology is also used. The mid-term storability, at 4°C, in darkness, of apricot ‘Pisana’ and apple ‘Cerina’ was evaluated. Shoots of ‘Pisana’ were multiplied in presence of equimolar amount of sucrose or sorbitol and benzyladenine or *meta*-Topolin and nodal segments from shoots were maintained, for 6 months, ‘naked’ or encapsulated in alginate, on media with 3%, 4.5% or 6% sucrose. Each 21 days, nodal segments from each treatment were transferred in standard growth conditions and re-growth was recorded after 21 days, as number of nodal segments developing shoots. After only 21 days the re-growth of explants multiplied on sucrose/benzyladenine was significantly lower than control, while for those multiplied on sorbitol/*meta*-topolin the re-growth reduction was significant only for encapsulation treatments and for the highest sucrose concentrations. However, the regrowth ability decreased linearly with the time for all treatments and the encapsulation had a detrimental effect on the re-growth. In ‘Cerina’ two sucrose concentrations (3.0 or 4.5%), salicylic acid (0.5 mM) or L-glutamine (5 mM) were applied. Shoots were stored in darkness at 4°C for 1, 3 and 6 months. At the end of each period the extension of necrosis in the shoots was evaluated and then shoots were transferred to standard growth conditions. After 21 days, multiplication rate and chlorophyll content were determined. The most effective treatment in limiting necrotic areas and the reduction of chlorophyll content was the supply of sucrose 4.5% in the medium. Salicylic acid significantly extended necrosis in explants, with a progressive increasing with the cold storage time, did not avoid reduction of chlorophyll content and induced a significantly increase of carotenoids content.

Keywords: encapsulation, in vitro culture, *Malus domestica*, mid-term storage, *Prunus armeniaca*, salicylic acid, sucrose concentration

S2-P60

MICROPROPAGATION Of *Aloe vera* L. via TEMPORARY IMMERSİON BIOREACTOR SYSTEM

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Aloe vera L. is an important medicinal perennial herb belonging to the family Liliaceae. The aim of this study was to investigate multiplication rate of in vitro shoots in temporary immersion bioreactor (TIB) system and to find appropriate immersion and ventilation periods. The principle of TIB system is simply immersing the plant material into liquid growth media for short periods of time. These immersions are sufficient for the plant’s nutrients uptake. TIB system profits from the advantages of liquid cultures, while growing the plants under high gas-exchange environments. Lateral shoots of greenhouse grown Aloe vera plants were used as explants. The explants harvested were washed carefully and kept in a chilled, sterile anti-oxidant solution (200 mg/l ascorbic

acid, 50 mg/l citric acid and 25 mg/l polyvinylpyrrolidone; PVP). These were surface-sterilizated with 70% ethanol for 2 min, 15 % sodium hypochlorite (commercial bleach) for 10 min, washed several times with sterile water and kept in chilled sterile antioxidant solution for 5 minutes. The shoots were cultured on Murashige and Skoog (MS) medium containing 4.4 µM 6-Benzylaminopurine (BAP), 30% sucrose and 6.5 g/l agar. After one month the shoots were transferred to TIB system (SETIS) for multiplication(10 shoots/container). The solid culture medium containing 4.4 µM BAP and 30% sucrose was found efficient for high multiplication rate in our earlier studies. Hence the same composition of the medium was used for liquid culture preparation. Immersion period was designed as twice a day (every 12 hours) for 5 minutes. Ventilation periods were designed six time a day (every 4 hours) for 45 minutes. In the present study wiht these periods maximum shoots number was found 14±4 per shoots . To reach the maximum shoot number on solid medium (27.8) different period designings are under planning.

Keywords: *Aloe vera*, micropropagation, SETIS

S2-P61

EFFECT OF H_2O_2 CONTENT AND ANTIOXIDANT ENZYMES ACTIVITIES DURING SOMATIC EMBRYOGENESIS OF THE MOROCCAN OLIVE CULTIVAR DAHBIA

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In vitro regeneration, through somatic embryogenesis from adult material, is possible with a limited tree species. To broaden our understanding of this process in olive (Olea europaea) tree, we investigate the evolution of some antioxidant enzymes during the stages of callus and embryos culture of the Moroccan olive cultivar 'Dahbia'. Hydrogen peroxide (H_2O_2) content and antioxidant enzymes activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) were evaluated during somatic embryogenesis of cultured leaf fragments, obtained from in vitro rejuvenated plantlets of 'Dahbia' cultivar. H_2O_2 content was higher in callus during the 6 weeks in induction phase, nevertheless this content gradually decreased when callus were transferred in expression phase. Activity of SOD gradually increases during somatic embryogenesis; and reached the maximum when somatic embryos were formed. However, POX and CAT activities were high in the early stages of somatic embryogenesis to decrease afterwards.

Keywords: somatic embryogenesis, hydrogen peroxide, catalase, peroxidase, superoxide dissmutase

S2-P62

SOMATIC EMBRYOGENESIS OF 9 MANGO (*Mangifera indica* L) GENOTYPES FROM CHINA

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Somatic embryogenesis is a powerful biotechnological tool which could be used for mass production of elite cultivars, genetic engineering and somatic hybridization. In this study, immature embryos derived from 9 core genotypes ('Jinhuang', 'Renong 1', 'Aimang', 'Guifei', 'Lingsheng', 'Rumang', 'Baixiangya', '063915', '9401') of China were cultured in vitro from April 2015 to April 2016. Significant differences were observed in the rate of callus induction of different genotypes. 'Linsheng' mango was the most responsive genotype for callus induction in MB5 medium (Wu et al., 2007) supplemented with 5 mg/L 2,4-D and 5 mg/L kinetin (KT), with the rate reaching 81.0% . However, only callus induced from 'Renong 1', 'Aimang', 'Guifei', 'Rumang' coud be able to further deveop into somatic embryos and seedlings, and the highest yield of somatic embryos (25.6/g) were produced from 'Guifei' embryogenic callus in MB5 medium. The highest seedling rate of somatic embryos (23.3%) were obtained from 'Guifei' cotyledon embryos in MB5 medium with 1 mg/L 6-BA.

Keywords: mango, somatic embryogenesis, genotypes, plantlet regeneration

S2-P63

IN VITRO PROPAGATION OF *Artemisia umbelliformis* subsp. *eriantha* INOCULATED BY ENDOPHYTIC PLANT-GROWTH PROMOTİNG BACTERIA

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Artemisia umbelliformis subsp. *eriantha* (Asteraceae) grows on limestone slopes exposed to the North and to cold winds up to 2300 m asl. It is an aromatic perennial herb, with relevant phytotherapeutic properties.All plants live in association with a wide and biodiversified microbioma that contribute to plant health and stimulate its growth at all stages, from seed germination to seed production. The microbioma is constitute by bacteria, archaea and fungi which produce plant growth promoting substances, auxin in particular, fix atmospheric nitrogen and hinder pathogen attack. In the present work, we describe a simple and efficient method for in vitro propagation of Artemisia umbelliformis subsp. eriantha using synthetic seed technology. Axillary buds of Artemisia isolated from aseptic multiple shoot cultures,w ere successfully encapsulated in calcium alginate beads with endophytic bacteria. The best encapsulation matrix was with 2% sodium alginate and 50mM CaCl2 (as complexing agent). The regrowth was evaluated in vitro on MS medium supplemented with 3% sucrose. In the present work, regrowth (defined as the number of induced plantlet in 4 weeks) was the parameter utilized to measure plantlet development. Differences in regrowth were observed after the treatments of the synthetic seeds, with or without endophytic bacteria. Not-inoculated encapsulated buds (control) showed after 4 weeks 60% plantlet development, while the inoculated 85%. In conclusion, synthetic seed technology would allow the production of Artemisia mass propagation material having the ability of regrowth, rooting and development increased by the presence of endophytic bacteria.Germplasm conservation is increasingly necessary for future sustainable harvesting systems and for maintaining species diversity to prevent genetic erosion. The alginate encapsulation technique and cryogenic procedures may be reliable methods for long-term storage of plant genetic resources.The research is ongoing, and will continuewith the sowing on a hydroponic system (ex vitro) of the synthetic-inoculated seeds.

Keywords: endophytic bacteria, encapsulation, shoots, synthetic seed, Artemisia

S2-P64

GROWTH AND DEVELOPMENT OF *Hakonechloa macra* (Mak.) IN TISSUE CULTURE DEPENDING ON THE TYPE AND COLOR OF LIGHT

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Hakonechloa macra (Hakone grass) is one of the most decorative ornamental grass. It produces arching leaves forming cascading mounds. It is propagated through division, however due to a slow growth it takes several years. Propagation of the species *in vitro* would allow to obtain a lot of plants in a relatively short time. The research on micropropagation of hakone grass was conducted in a tissue culture laboratory of the Department of Ornamental Plants, Dendrology and Landscape Architecture of the University of Life Sciences in Lublin, Poland. The aim of the presented studies was to estimate growth and development of *Hakonechloa macra* plants cultivated *in vitro* depending on the type and color of light. The plant material were 2 cm tufts derived from a stabilized tissue culture, which were placed on a Murashige and Skoog (MS) medium [1962], supplemented with benzyladenine (BA) 3 mg·dm^{-3} and indolebutyric acid (IBA) 0.3 mg·dm^{-3}. The media pH was 5.7. The explants were cultivated at 16-hour photoperiod, under fluorescent white light (W) or different color combinations of LEDs (1 blue with filter + 11 red, 1 green + 1 blue + 10 red, 2 green + 1 blue + 9 red, 1 blue + 11 red, 3 red + 1 blue + 7 white).The light intensity was 35 µmol·m^{-2}·s^{-1}. The obtained results were evaluated statistically. It was

estimated that the light type and color influenced growth and development of *Hakonechloa macra in vitro*. There were no differences in the number of progeny tufts, however, the number of tufts per explant was definitely lower under white fluorescent light or under LED light obtained from 1 blue with filter and 11 red diodes.

Keywords: LEDs, fluorescent light, micropropagation, multiplication, rooting

S2-P65

IN VITRO PROPAGATION OF *Anadenanthera colubrina* (Vell.) BRENAN

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Anadenanthera colubrina (Vell). Brenan is a Brazilian native forest species with arboreal size, used in the afforestation of pastures, wood, coal and in tanneries, due to the high tannin content of its bark. The application of in vitro cultivation techniques prior to optimize the propagation of native forest species plays an important role in the scientific research of potentially economical and diversified plants. The objective was to study in vitro germination, shoot induction and acclimatization of A. colubrina embryonic axes. Embryonic axes were exposed to different times (5, 10 and 15 min) of sodium hypochlorite (2.0 and 2.5% active chlorine) and paraformaldehyde 80% (5, 30, 60 and 120 min) for disinfestation. In vitro germination of embryonic axes was evaluated for the effects of different GA3 (0.0, 2.0, 4.0, 6.0 and 8.0 mg L^{-1}), BAP (0.0, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}) and culture media (MS and WPM). In order to obtain shoots, stem segments from in vitro seedlings were inoculated in WPM medium, supplemented with different concentrations of BAP (0.0, 1.0, 2.0, 4.0, 8.0 and 10.0 mg L^{-1}) and ANA (0.0, 0.01, 0.1 and 1.0 mg L^{-1}), 30.0g L^{-1} sucrose and 7.0g L^{-1} agar, pH was corrected to 5.8. After the inoculation, the explants were kept in a growth room with a mean temperature of 25 °C, under a photoperiod of 16 hours and irradiance of 36 µmol m-2 s-1. For rooting, the WPM culture medium was supplemented with 30.0 g L^{-1} sucrose, 7.0 g L-1 agar, 0.1% activated charcoal and different concentrations of AIB (0.0 , 1.0, 2.0, 5.0, 8.0 and 10.0 mg L^{-1}) and hydrolyzed casein (0.0, 0.5, 1.0, 2.0, 4.0 g L^{-1}). Subsequently, shoots were individualized and acclimatized with Tropstrato® substrate in greenhouse, and the percentage of survival was evaluated 30 days after acclimatization. Data were submitted to analysis of variance (ANOVA) and according to results the qualitative data were compared by the Tukey test ($p<0.05$), and the quantitative data were analyzed by polynomial regression ($p<0.05$). The use of paraformaldehyde for 120 min is efficient in disinfestation of embryonic axes. The culture media tested are efficient for in vitro germination of embryonic axes. The use of BAP promotes efficient response in shoots induction. IBA and hydrolyzed casein at the concentrations tested were not efficient for the induction of rhizogenesis in shoots. Seedlings of A. colubrina presented, after 30 days in a growth room, only 9% of survival, even being submitted to a pre-acclimatization. This low survival rate shows the fragility of A. colubrina tissues, especially callus formation in the roots of in vitro seedlings, possibly hindering its establishment in vitro. The presence of stomata was verified in the adaxial and abaxial epidermis of in vitro seedlings, which characterizes the amphistomatic species. There was no statistical difference for the stomatal density, equatorial and polar diameter of the stomata on both epidermal faces of the in vitro seedling leaflets. Support: CAPES, FAPEMIG and CNPq.

Keywords: angico-vermelho, fabaceae, forest species

S2-P66

IN VITRO PROPAGATION OF *Zehneria platysperma* (W.J. de Wilde & Duyfjes) H. Schaef. & S.S. Renner (CUCURBITACEAE), AN ENDEMIC PLANT OF THAILAND

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Genus *Zehneria* is a member of plant in Cucurbitaceae that contains glycosides and sphingolipids at its roots and leaves. Some species of *Zehneria* is used as local folk medicine for curing skin diseases. It also shows antimicrobial and antitumor activities. In Thailand, *Zehneria platysperma* (Cucurbitaceae) is classified as an

endemic plant which is found only in Northern and South Western regions. This plant may have risk in extinction due to deforestation problem. Therefore, this study established an in vitro propagation protocol for conservation of *Z. platysperma*. Detached seeds were sterilized surface using sodium hypochlorite (NaOCl) and inoculated onto Murashige and Skoog (MS) agar medium for initiating the in vitro clean culture. Sixteen-week-old seedlings were selected for using as plant materials. The first and second nodes from base of seedling were excised and used as explants. Nodes were cultured on MS agar medium supplemented with different concentrations of N^6-benzyladenine (BA; 0-4 mg/L). Then, regenerated plantlets were transferred onto BA-free MS agar medium. At the 8th weeks after culturing the nodes, the highest shoot numbers (9.30±0.34 shoots/explant) with an average shoot height at 9.45±0.25 cm was obtained from *Z. platysperma* that had been cultured on MS agar medium supplemented with 1 mg/L BA. This medium also provided an average root numbers per explant at 12.40±2.28 with the longest root length (20.67±1.83 cm). Increasing of BA concentration in culture medium reduced shoot and root regeneration. Moreover, hyperhydric symptom was found from explants that cultured on 2 and 4 mg/L of BA. In addition, flowering and fruiting were found during in vitro condition. Vigorous plantlets with well-developed roots survived in natural condition after acclimatization process. This presented protocol can be a useful resource and tool for rapid proliferation and conservation *Z. platysperma*. Furthermore, this protocol may provide an authentic material for future phytochemical or medical studies.

Keywords: *Zehneria platysperma*, cucurbitaceae, endemic plant, in vitro propagation, N^6-benzyladenine

S2-P67

DEVELOPMENT OF CRYOPRESERVATION PROTOCOL FOR BANANA VARIETY "AMBUL" (*MUSA* spp.) USING VITRİFİCATİON TECHNIQUE

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Banana has a diverse germplasm and the cultivar 'Ambul-Nadee' (*Musa acuminate* spp. AAB) is one of the most popular among Sri Lankans which can be grown in most agro-ecological zones. The variety is released by the Department of Agriculture Sri Lanka in 1992 for general cultivation. The presence of characteristic flavor, aroma and the small size inherits a great potential for increased production to serve the export market. According to the National Red List of Sri Lanka, 2012 'Gal Kesel', 'Unel', 'Ati kesel', cultivars are categorized as critically endangered or endangered species. Therefore, their preservation is essential for future plant breeding and crop improvement programs. Cryopreservation has been introduced as a new technique of conservation of germplasm which is an important tool for long-term storage of plant genetic resources. This overcomes the problems related to sub culturing which reduces the time and the labor requirements needed and eliminate the somaclonal variations. This technique is newly introduced to Sri Lanka by Plant Genetic Resources Centre (PGRC) and *in-vitro* accessions are not found in the cultivar 'Ambul-Nadee'. Therefore, this study was focused on developing a cryopreservation protocol using *in-vitro* derived shoot meristems by vitrification technique considering the thawing temperature, thawing time and regeneration medium. Thawing temperature 40 °C and 42 °C were tested and one minute and two minutes thawing times were applied. For the regeneration medium Murashige and Skoog (MS) medium supplemented with 17.7 µM 6-Benzylamionpurine (BAP) and the second regeneration medium was prepared with 10.2 µM of BAP and 10.2 µM of Indole-3-acetic acid (IAA) with MS medium. After four weeks of culture period the number of survived, blackened and contaminated banana meristems were counted and recorded. With tested chemical and physical conditions, thawing temperature 42 °C was significant.

Keywords: cryopreservation, banana, *in-vitro*, germplasm, meristem, thawing, germplasm

S2-P68

PRODUCTION OF VIRUS-FREE APPLE ROOTSTOCK BY THERMOTHERAPY, CHEMOTHERAPY AND SHOOT TIP CULTURE

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Apple (*Malus domestica*) is one of the most important fruits worldwide. But virus diseases have threatened sustainable production of apple and caused the serious problems such as graft incompatibility, reduction of growth vigour, yield loss and poor fruit quality. Virus or viroid infection including Apple chlorotic leaf spot virus, Apple stem pitting virus, Apple stem grooving virus, Apple mosaic virus and Apple scar skin viroid has been also reported in Korea, furthermore, its damages and economic losses have increased constantly. In our studies, we attempted to eradicate major apple tree viruses from apple rootstock 'M.9' and 'M.26' using thermotherapy, chemotherapy and shoot tip culture for efficient production of healthy and virus-free plants. After treatments, the detection of apple viruses were accomplished by RT-PCR and ELISA. Efficiency of virus elimination was enhanced up to 60% in shoot tip culture, however, lowest at 10~20% after chemotherapy using antiviral agent (ribavirin). Detection accuracy of RT-PCR method was 30% higher than that of ELISA. Most apple rootstock samples collected from main distribution markets were verified to have multiple infection. From these results, we can suggest that combination treatment of shoot tip culture and thermotherapy may be more effective for the elimination of apple viruses from apple rootstock.

Keywords: apple, rootstock, virus elimination, heat treatment, ribavirin, in vitro culture

S2-P69

IMPROVMENT OF SOMATIC EMBRYOGENESIS IN WOODY SPECIES USING TIS PLANTFORM BIOREACTOR

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The aim of this work was to improve and increase somatic embryo formation, maturation and germination using partial desiccation treatment. To achieve this, embryogenic callus was transferred to either sealed test tubes or a Plantform bioreactor, without medium or aeration, for different number of days. After desiccation embryogenic callus was transferred to a regeneration medium for 16 weeks. During the study it was found that embryogenic callus subjected to desiccation in both test tubes and sterile bioreactor increased significantly the numbers of somatic embryo formation and germination compared to control treatment. Addition, transfer of mature and germinated embryos to TIS (temporary immersion system) system containing 500 ml liquid MS medium and 0.1mg/L Benzyl amino purine, 0.1 2ip and 0.1 Kinetin (KN) and high level of sucrose (100gm/L) induced a perfuse numbers of somatic embryos.

Keywords: Plantform bioreactor, desiccation, callus, somatic embryo, maturation, *in vitro*, TIS system, *Phoenix dactylifera* ,

S2-P70

A NEW MICROPROPAGATION PROTOCOL FOR *Rheum rhabarbarum* L. AND ANALYSIS OF GENETIC FIDELITY OF IN VITRO DERIVED PLANTS

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An efficient micropropagation method for *Rheum rhabarbarum* L. (Romanian genotype) was developed in this study. For in vitro culture initiation we used buds from rhizomes harvested from the field at the end of March. In all the multiplication stages, modified Murashige and Skoog (1962) media were used, coded as MSm: PGR-free MSm in the initiation stage as well as in the in vitro rooting stage, MSm supplemented with 6-benzyladenine (0.5 mg L^{-1}; 2 mg L^{-1} and 4 mg L^{-1}) for the in vitro culture stabilization stage and MSm supplemented with 4 mg L^{-1} 6-benzyladenine; 4 mg L^{-1} kinetine; 4 mg L^{-1} 2-isopentenyladenine; 4 mg L^{-1} meta - topoline in the

multiplication stage. The rhubarb microplants obtained in the multiplication stage (the proliferation rates were 4.90±0.51) were rooted in vitro – 96% rooting percentage and acclimatized ex vitro in floating perlite, with 90% acclimatization percentage. After acclimatization the plants were transferred to a peat-based potting mix - 98 % survival percentage and in six months 2-3 cm long rhizomes developed. To assess the genetic fidelity between derived plants and the mother plant, sequence related amplified polymorphism (SRAP) markers were used. Out of 12 SRAP primer combinations, only 8 primer pairs produced reproducible and scorable bands. All banding profiles from the micropropagated plants were monomorphic and similar to those of the mother plant indicating 100% similarity. This confirmed the true-to-type nature of the rhubarb plantlets obtained in vitro.

Keywords: growth regulators, subculture, SRAP, genetic fidelity, rhubarb

S2-P71

BIOCHEMICAL ANALYSIS ON THE GERMINATION OF *Passiflora ligularis* ZYGOTIC EMBRYOS AFTER CRYOPRESERVATION

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Cryopreservation is a process where live biological structures are preserved by cooling to very low temperatures (−196 °C using liquid nitrogen). Among cryopreservation techniques, PVS2 vitrification by the use of Plant Vitrification Solution 2 - PVS2 for long periods can interfere in cellular homeostasis. Therefore, this study aims to evaluate the influence of exposure time to the cryoprotectant PVS2 on the mobilization of reserves and on antioxidant metabolism during the germination of cryopreserved Passiflora ligularis zygotic embryos. The centesimal composition was analytically determined for P. ligularis seeds. Cryopreservation procedures consisted on test different exposure times to PVS2 (0, 30, 60 and 120 minutes) for zygotic embryos, after 30 days of in vitro culture, the percentage of germination and germination speed index (GSI) were evaluated. Proline content, hydrogen peroxide, activity of isocitrate lyase (ICL), malate synthase (MSy), lipidic peroxidation and antioxidant enzyme activity (SOD, CAT, APX) were evaluated on zygotic embryos at 7, 14 and 21 days after cryopreservation. P. ligularis seeds presented a large percentage of lipids (31%) in their reserves, characterized as oil seeds. The germination from cryopreserved zygotic embryos increased significantly and reached the maximum (85%) with GSI (0.6) after 60 min of PVS2 exposure. The highest values for the activity of the exclusive enzymes of the glyoxylate cycle, ICL and MSy were recorded after 60 min of exposure to PVS2 solution, accelerating the reserve mobilization and consequently the germination process. The increase of proline content, as well as the activity of antioxidant enzymes (SOD, CAT, APX) and a decrease of lipidic peroxidation was also verified, which was able to optimize the long-term conservation of this species. Plants of P. ligularis was completely regenerated 60 days after cryopreservation. In conclusion, the application of the cryopreservation technique, in addition to being a tool for conservation, can be applied to accelerate and standardize germination and also may have long-term practical importance for oilseeds. Acknowledgment: CNPq, CAPES and FAPEMIG.

Keywords: *Sweet granadilla*, long-term storage, cryopreservation, glyoxylate cycle, antioxidant metabolism

S2-P72

EVALUATION OF THE GENETIC FIDELITY OF IN VITRO-PROPAGATED BLACKBERRY (*Rubus fruticosus* L.) USING MOLECULAR MARKERS

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The aim of this research was to evaluate the genetic uniformity of blackberry planting material (*Rubus fruticosus* L., cultivars ‘Loch Ness’ and ‘Chester Thornless’) obtained by micropropagation. Genetic uniformity was analyzed by RAPD and SRAP markers. For in vitro multiplication, the slightly modified Murashige and Skoog (1962) basal medium was used, supplemented with 0.5 mg L^{-1} 6-Benzyladenine (BA), prepared with tap water

and 50 g/l wheat starch as gelling agent. This culture medium ensured the regeneration of well-developed plantlets, with multiplication rates of more than 42 for both cultivars. In vitro multiplication was carried out in 30 months including 12 subcultures. The plants obtained from the 3rd and 11th subcultures were compared with the mother plants using 64 SRAP primer combinations (eight forward and eight reverse primers) and 20 RAPD primers. The amplification products were monomorphic in the micropropagated plants and similar to those of the mother plant. No polymorphism was detected, thus proving the genetic fidelity and uniformity of the micropropagated plants.

Keywords: 'Chester', 'Loch Ness', SRAP, RAPD, tissue culture

S2-P73

FACTORS AFFECTING BULBLET GROWTH OF *Lilium* sp.'A CORRELATIVE ASSESSMENT OF CULTURE MEDIUM IN VITRO

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The lily is an excellent model for studying the correlation between culture media and bulblet growth to provide a fundamental relationship for bulblet production *in vitro*. Bulblet growth is the most important factor in understanding how lily bulblets regenerate and grow *in vitro* and the role of media on the growth mechanism in lilies. The aim of this study was to examine the effect of different culture media on enhancing bulblet size and gain more information about bulblet production *in vitro*. Hence, solid versus semi-solid versus liquid static culture were investigated. The results show that explants grown in liquid medium formed bulblets that had the best fresh weight, bulblet number, and root:shoot ratio compared with explants grown in semi-solid and solid media. Furthermore, a linear relationship amongst the culture media irrespective of cultivar suggests culture medium significantly influences lily explant regeneration and bulblet production *in vitro*. The results of bulblet performance including the root:shoot ratio indicate that bulblet growth in a liquid static culture was sustained by the renewal of liquid medium, thus avoiding nutrient depletion, and might be a useful technique for producing bulblets and enhancing their growth *in vitro* compared with bulblets grown on an agar solidified medium.

Keywords: *Lilium* sp.; bulblet growth; correlation; culture media; *in vitro*

S2-P74

ESTABLISHMENT OF A PAPAYA MICROPROPAGATİON PROTOCOL (*Carica papaya* L.) IN A TEMPORARY TWIN VESSEL IMMERSION SYSTEM (SIT)

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Papaya production is affected due to sexual variability in the seed. The methods of conventional propagation involve the maintenance of large land plantations to supply the consumer's demand. An alternative is micropropagation using bioreactors of temporary immersion of twin vessels (BIT). The purpose of this work was the establishment of a disinfection methodology for the introduction of axillary buds and the evaluation of immersion time and inoculum density in the BIT to obtain a higher multiplication rate. Disinfection of the plant material was performed with 70% ethanol for 1 minute, 2% sodium hypochlorite for 5, 6 and 8 min, and washed with sterile distilled water. For the introduction, Murashige & Skoog medium was used with 1 $mg.L^{-1}$ of gibberellic acid and 2 $mg.L^{-1}$ of kinetin; in the same way for multiplication supplemented with 0.5 $mg.L^{-1}$ of

benzyl adenine, 0.5 mg.L^{-1} of indoleacetic acid and 0.3 mg.L^{-1} of gibberellic acid. For 21 days the multiplication rate, number of leaves, height and diameter of the stem was evaluated. The plant material was transferred to the BIT to evaluate the immersion time (1 and 2 min) and density of the inoculum (4 and 8) in 200 mL of liquid multiplication medium, with an immersion frequency of 6 h, photoperiod of 12 h of lighting per day and 28ºC. In the disinfection with 2% sodium hypochlorite for 6 and 8 min, 100% survival without oxidation was achieved. In the introduction stage, 96.4% survival was obtained; similarly in the stage of multiplication in semi-solid medium (83.33%), with a number of leaves 1.88 ± 0.02, height 1.12 ± 0.04 cm, diameter of 0.43 ± 0.01 cm and a multiplication rate of 1.20 ± 0.02 . In the BIT, in a time of 2 min of immersion every 6 h with an inoculum density of 8, a number of leaves was obtained 6.03 ± 0.04, height 1.65 ± 0.10 cm, diameter 0.73 ± 0.02 and a multiplication rate of 5.05 ± 0.06. This work in addition to generating an academic contribution, could contribute to the strengthening of the productive sector, offering seedlings with the suitable sex (hermaphrodite), and high genetic and phytosanitary quality.

Keywords: papaya, in vitro propagation, yemporary immersion systems

S2-P75

ELEVATED AUXIN AND REDUCED CYTOKININ CONTENTS IN ROOTSTOCKS IMPROVE THEIR PERFORMANCE AND GRAFTING/MICRO-GRAFTING SUCCESS

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Plant grafting/micro-grafting is an important technique for horticultural and silvicultural practice. However, many rootstock plants suffer from undesirable lateral bud outgrowth, low grafting success rates or poor rooting. Here, we used a root-predominant gene promoter (SbUGT) to drive the expression of a tryptophan-2-monooxygenase gene (iaaM) from Agrobacterium tumefaciens to increase sauxin levels in tobacco. The transgenic plants, when used as a rootstock, displayed inhibited lateral bud outgrowth, enhanced grafting success rate and improved root initiation. However, root elongation and biomass of SbUGT::iaaM transgenic plants were reduced compared to those of wild-type plants. In contrast, when we used this same promoter to drive CKX (a cytokinin degradation gene) expression, the transgenic tobacco plants displayed enhanced root elongation and biomass. We then made crosses between the SbUGT::CKX and SbUGT::iaaM transgenic plants. We observed that overexpression of the CKX gene neutralized the negative effects of auxin overproduction on root elongation. Also, the simultaneous expression of both the iaaM and CKX genes in rootstock did not disrupt normal growth and developmental patterns in wild-type scions. Our results demonstrate that expression of both the iaaM and CKX genes predominantly in roots of rootstock inhibits lateral bud release from rootstock, improves grafting and micro-grafting success rates and enhances root initiation and biomass.

Keywords: auxin, cytokinins,rootstock, root growth anddevelopment, lateral buds release,grafting and micro-graftingsuccess rate

S2-P76

MİCROPROPAGATION OF MEDITERRANEAN AND EXOTIC SHRUBS: PROTOCOLS FOR ENDANGERED AND HIGH-VALUE PLANT SPECIES

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Sicily (south Italy) is a Mediterranean region characterized by mild winters and hot dry summers: it is defined as a hotspot of plant biodiversity, moreover several species native from other countries have been easily naturalized. Since 1999, the CREA - Research Centre for Plant Protection and Certification of Palermo (formerly

known as Research Unit for Mediterranean Flower Species) has been involved in national and regional research projects aimed to the conservation of the autochthonous biodiversity (i.e. rare and endangered species, high-value genotypes) for landscaping and functional uses as well as to the introduction of new exotic germplasm for ornamental scopes and breeding. Therefore, numerous spontaneous (Crataegus monogyna, Erica multiflora, Genista aetnensis, Hypericum perforatum, Myrtus communis, Pistacia lentiscus) and exotic (Euphorbia x lomi, Hibiscus rosa sinensis, Metrosideros excelsa, Oreopanax capitatus) shrubs have been introduced and propagated in vitro using different techniques (bioreactors, magnetic fields, micrografting, regeneration, etc.). For each species, an efficient micropropagation protocol has been defined. In the present paper, the used culture media, plant growth regulators, acclimatization substrates and main results (in terms of aseptic explants, shoots and roots emission, acclimatized plantlets), obtained during sterilization, multiplication, proliferation, rooting and acclimatization, are briefly described.

Keywords: in vitro propagation, culture media, plant growth regulators, plant biodiversity

S2-P77

COFFEE SYNTHETIC SEEDS PRODUCTION IS INFLUENCED BY SOMATIC EMBRYO DEVELOPMENTAL STAGE AND NUTRITIONAL FACTORS ASSOCIATED TO THE ENCAPSULATION MATRIX

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Plant propagation through artificial seeds has opened new possibilities in agriculture, through applications in the in vitro plant propagation and short- to medium-term germplasm conservation. The current propagation method of *Coffea arabica* is mainly based on seeds, which brings disuniformity to coffee tree plantations, therefore, synthetic seed technology in an association with large-scale somatic embryos production could be an alternative to render more efficient coffee plant propagation. This work demonstrates the influence of the somatic embryos developmental stages and nutritional factors associated to synthetic coat on production of artificial seeds for the propagation of *C. arabica*. Induction of somatic embryos was favored by more than 40% in liquid medium MGM compared to the solid. The use of 2, 4 or 8% (w/v) sodium alginate with 100 mM calcium chloride (CaCl2·2H2O) leads to isodiametric artificial seeds formation. The employment of somatic embryos at less developed stages decreased the germination frequency. The best germination frequency (77.5%) of artificial seeds was achieved by the use of somatic embryos with early developed radicle and alginate-encapsulated with DEV medium (MS salts with 0.01 mg.L-1 of biotin, 100 mg.L-1 of myo-inositol and 0.3 mg.L-1 of benzyl-amino-purine) associated to the encapsulation matrix, later germinated in same medium. To the best of the knowledge, this is the first report on synthetic seed production in *C. arabica*, therefore an important step forward the acquisition of a better propagation system for this specie.

Keywords: *Coffea arabica*, somatic embryogenesis, clonal propagation

S2-P78

ESTABLISHMENT OF MASS PRODUCTION SYSTEM OF VIRUS-TESTED APPLE ROOTSTOCK *(Malus domestica)* M9 USİNG AIR-LIFT BIOREACTOR

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Apple (*Malus domestica*) is required to have a virus-tested plant mass production system to meet the demand of the domestic fruit tree industry because the quality and yield of the plant is reduced by 30 ~ 50% when the virus is infected. Therefore, in this study, to obtain virus-tested plants and to mass-produce them, co-treatment of shoot-tip culture (1 mm size) with 32 ℃ heat treatmen
the 1st experiment, obtained virus-tested plants were cultured in an air-lift bioreactor with different culture system by raft culture and temporary immersion (TIB) culture. The immersion time in the transient immersion

system was 1 time per 3 hours (TIB-3) and 6 times per 6 hours (TIB-6), and the control was a solid culture and liquid stagnation culture. The fresh weight of the plant was 182.6 mg in TIB-3 treatment, and the length of the top part and the bottom part was about 2 times and 1.6 times longer than that of the solid culture, respectively. The rate of plant development with roots was the best at 100% in TIB-6, and the rate of planting in liquid medium was the lowest at 45.4%. The leaf area was the smallest in the plants grown in the solid culture medium (control). The plants harvested from bioreactor system did not show hyperhydricity in all treatments. Even though the growth rate of the plant was good in the bioreactor, but the production rate was about 59 ~ 75%, which is lower than that of the control (97 ~ 100%), and it should be improved in further study. This study suggests that the air-lift bioreactor can produce a large number of virus-tested plants in a short time compared with the conventional culture system. Additionally, the secondary xylem of the stem is well developed and thus it shows the possibility to obtain healthy plants which are suitable for the acclimatization.

Keywords: bioreactor, apple, rootstock, in-vitro, micropropagation.

S2-P79

CYTOLOGICAL CHARACTERISTICS OF CALLUS AND ADVENTITIOUS ROOTS DERIVED FROM VARIOUS EXPLANTS AND THEIR PHENOLICS PROFILING BY HPLC IN *Camellia japonica*

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Camellia japonica is an evergreen tall tree belonging to Theaceae, and enrich in physiologically bioactive compounds such as anthocyanin, catechin, tannins A, and B. Recently, the plant has been growing in demand for biomaterials in the cosmetic industry. This study was conducted to establish in vitro culture condition of the callus and adventitious roots from various types of explants, and analyzed their bioactive compounds in Camellia japonica. The various combinations of growth regulators were tested to induce callus from petal in two cultivars ‘Seohyang’ and ‘Black lace’ of C. japonica. And also callus and adventitious roots (AR) were induced from four different explants (leaf, root, petal, and ovary) in the selected medium. Their cell division was analyzed by flow cytometry, and 17 phenolic compounds were analyzed by HPLC. The proliferation rate was the highest in ovary-derived callus (OC) with 2.1 times (4.1 g FW/ ea) than that of leaf-derived callus (LC) which is the lowest one. The OC was also the highest in dry weight, 1.2 times (165mg/ ea) higher than the lowest petal-derive callus (PC). In adventitious roots, the highest fresh weight and dry weight were achieved in the leaf-derived adventitious root (LR), and interestingly it was opposite with callus result that LC resulted in the lowest proliferation rate in FW and DW. Profiling of 17 phenolics on each line of calli and ARs revealed that more phenolics were analyzed in the ARs than in the calli because the ARs were composed of more diverse cells due to the differentiation in terms of development. When compared by origin, it was analyzed that the content was highest in callus and adventitious root derived from the ovary. The results abstained here showed that ARs contained various phenolics, and it can be used as one of the antioxidant cosmetic substances.

Keywords: micropropagation, in vitro, *Camellia japonica*, secondary metabolites, explants type

S2-P80

IN VITRO MICROPROPAGATION OF MOSSES *Taxithelium kerianum* AND *Leucobryum aduncum*

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Mosses are the second largest terrestrial plants, yet the information of ethno-bryology and the application of mosses as medicines are very limited. Therefore, this study was designed to optimize in vitro propagation of the selected mosses Taxithelium kerianum and Leucobryum aduncum. For this, both spore of T. kerianum and gametophyte explants of L. aduncum were surfaced sterilized by using fungicide, NaClO, cloroxylenol, ethanol and HgCl2. Subsequently, these explants were grown in either Murashige and Skoog (MS) or Knop medium supplemented with napthalene aceticacid (NAA), 3-indole aceticacid (IAA), kinetin, zeatin, and gibberelic acid (GA3). The results showed that cloroxylenol 0.48% (10 min)+ ethanol 70% (5 sec) + HgCl2 0.025% (5 min) were effective to sterilize T. kerianum spores, while cloroxylenol 0.48% (5 min) + ethanol 70% (5 sec) + HgCl2 0.025% (5 min) were effective for L. aduncum gametophore sterilization. Furthermore, growth of both species

were significantly better in Knop medium compared to basal MS medium. The addition of growth hormones also resulted in different responses on the development of protonema from T. kerianum. IAA induced the new formation of protonema filaments, kinetin induced gametophores branching, while GA3 induced stem elongation and shoot multiplication. In contrast, none of these hormone treatments influenced growth and development of L. aduncum gametophore. The obtained results will be critical not only to study the development of different moss spesies, but also to provide sufficient biomass of the selected mosses for various purposes.

Keywords: *Leucobryum aduncum*, mosses, micropropagation, *Taxithelium kerianum*

S2-P81

ROOTING AND EX VITRO ADAPTATION OF MICROPROPAGATED PLANTS OF CHILEAN STRAWBERRY *Fragaria chiloensis* (L.) Duch

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The Chilean strawberry (*Fragaria chiloensis* L. Duch) is a berry fruit of great agricultural and commercial potential due to its excellent organoleptic properties, its exquisite aroma and flavour, and the exotic white/pink colour of its fruits. Vegetative propagation of Fragaria sp. is traditionally carried out using stolons. This system of propagation, in addition to being slow, can spread several plant diseases, mainly viral. In vitro culture of meristems and the establishment of micropropagation protocols are important tools for solving these problems. In recent years, considerable effort has been made to develop in vitro propagation of commercial strawberry cultivars in order to produce virus-free plants with high quality. These previous results can serve as the basis for developing in vitro-based propagation technologies in the less studied species Fragaria chiloensis. The ex vitro adaptation of plants is a key step during micropropagation of plants, determining sometimes the economical and technical efficiency of the technology. Rooting and hardening of the micropropagated plants still under the in vitro conditions can improve the ex vitro efforts. In this study, the effect of different auxin treatments, sucrose concentration and flask covers on the efficiency of plant recovery during the ex vitro step is informed. The results showed that shooting and rooting during under ex vitro conditions is influenced by IBA and NAA and the genotype. Plant height is influenced only by auxin treatments. Sucrose addition (5%) improved the development of new shoots and roots under in vitro conditions and gave higher plant survival during ex vitro adaptation.

Keywords: micropropagation, ex vitro adaptation, rooting auxin

S2-P82

GRAPEVINE CALLOGENESIS AND MICROPROPAGATION PROTOCOL FROM SHOOTS AND LEAF EXPLANTS

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The increasingly decisive role of genetic engineering in the breeding of plants, especially in light of in progress climate change, requires an efficient regeneration method for vegetatively propagated species, such as the grapevine. This work represents the preliminary phase to set up an efficient and high-throughput protocol to obtain calli and vine plantlets of M4, 1103 P, 101.14 rootstocks, to be transformed with the gene considered to be the putative factor of the M4's better adaptation to drought condition. To reach this goal the genome editing technique -particularly the type II CRISPR/Cas9 system– will be used. Greenhouse-grown shoots were surface-sterilized for 10 minutes in a solution containing sodium hypochlorite, diluted in sterile distilled water, and a few drops of Tween 20 detergent. Afterwards, they were washed another 3 times for 5 minutes in sterile distilled water. Working under a fume hood, shoots were cut into one-node segments and their leaves were removed. Segments were cultured on a root-inducing medium composed of half-strength Murashige and Skoog (MS) salts

and vitamins, added with 0.1 mg/l IBA and 0.5 mg/l BAP; while bisected leaves, on a callus-inducing MS medium with 1.0 mg/l BAP and 0.1 mg/l 2,4-D. Both media were supplemented with 30 g/l sucrose and 8 g/l agar, adjusted to pH 5.8 and autoclave at 121°C for 20 minutes. One-node segments were incubated at 23±1°C with a 16-hr photoperiod; leaves in the dark until the callus appears. After a week, regenerating shoots were transferred to MS media without growth regulators for adventitious shoot formation. Calli were sub-cultured every 4 weeks and different adventitious shoot-inducing media were used to regenerate plantlets. The different attitude of each rootstock to obtain calli and vine plantlets will be described.

Keywords: in vitro culture, micropropagation, callogenesis, roostock

S2-P83

GRAPEVINE REGENERATION AND MICROPROPAGATION PROTOCOL FROM SHOOTS AND LEAF EXPLANTS

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The increasingly decisive role of genetic engineering in the breeding of plants, especially in light of in progress climate change, requires an efficient regeneration method for vegetatively propagated species, such as the grapevine. This work represents the preliminary phase to setting up an efficient and high-throughput protocol to obtain calli and vine plantlets of M4, 1103 P, 101.14 rootstocks, to be transformed with the gene(s) considered to be the putative factor of the M4's better adaptation to drought condition. To reach this goal the genome editing technique -particularly the type II CRISPR/Cas9 system– will be used. Greenhouse-grown shoots were surface-sterilized for 10 minutes in a solution containing sodium hypochlorite, diluted in sterile distilled water, and a few drops of Tween 20 detergent. Afterwards, they were washed another 3 times for 5 minutes in sterile distilled water. Working under a fume hood, shoots were cut into one-node segments and their leaves were removed. Segments were cultured on a root-inducing medium composed of half-strength Murashige and Skoog (MS) salts and vitamins, added with 0.1 mg/l IBA and 0.5 mg/l BAP; while bisected leaves, on a callus-inducing MS medium with 1.0 mg/l BAP and 0.1 mg/l 2,4-D. Both media were supplemented with 30 g/l sucrose and 8 g/l agar, adjusted to pH 5.8 and autoclave at 121°C for 20 minutes. One-node segments were incubated at 23±1°C with a 16-hr photoperiod; leaves in the dark until the callus appears. After a week, regenerating shoots were transferred to MS media without growth regulators for adventitious shoot formation. Calli were sub-cultured every 4 weeks and different adventitious shoot-inducing media were used to regenerate plantlets. The different attitude of each rootstock to obtain calli and vine plantlets will be described.

Keywords: in vitro culture, vitis spp, rootstock, callogenesis