

# Fruit, Vegetable and Cereal Science and Biotechnology

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- 2) Mycorrhizal symbioses (and effects on plant physiology, productivity, reproduction and disease resistance);
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**Cover photos:** Top left and right: Field-grown and tissue-cultured taro in Saipan (Nandwani, pp 38-43); Top, center: GUS expression in transformed hypocotyls of okra (Mallela *et al.*, pp 1-6); Bottom right corner: Appearance of leaves of tomato plants grown under control, water stress, salinity stress, and combined stress conditions (Xu *et al.*, pp 54-61); Bottom, left: *In vitro* rooting of 'MM111' apple (Ali Bacha *et al.*, pp 28-34). Bottom, center: Multiple shoot development from okra cotyledonary nodal meristems on MS medium with BAP (1.0 mg/L) (Mallela *et al.*, pp 1-6).

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## Fruit, Vegetable and Cereal Science and Biotechnology

**Rekha Rani Mallela, Prathap Reddy Vutukuri, Manorama Kanuri (India)** *In Vitro* Plant Regeneration and Genetic Transformation of Okra (*Abelmoschus esculentus* [L.] Moench) (pp 1-6)

### ABSTRACT

**Original Research Paper:** Regeneration of recalcitrant okra (*Abelmoschus esculentus* [L.] Moench) plants *in vitro* would permit their rapid propagation and also aid in genetic transformation. Explants (hypocotyls, cotyledonary nodal meristems, cotyledons and shoot tips) were excised from okra seedlings that were grown aseptically in Murashige and Skoog (MS) basal nutrient medium. The explants were cultured on MS medium supplemented with auxins, cytokinins, and different auxin-cytokinin combinations. More organogenic callus was obtained on medium fortified with MS nutrients with low concentrations of 2,4-D (dichlorophenoxy acetic acid), NAA ( $\alpha$ -naphthalene acetic acid) and TDZ (thidiazuron), in combination. Shoots were produced on hypocotyls, cotyledonary nodes, and shoot tips, and calli were derived from leaf and cotyledon explants cultured in medium supplemented with BAP (6-benzylaminopurine) and TDZ. Both Kn (kinetin) and Zn (zeatin) proved ineffective in inducing either shoot buds or shoots. Roots were induced on elongated shoots using MS medium containing NAA or IAA (indole-3-acetic acid). Genetic transformation was carried out with *Agrobacterium tumefaciens* carrying the plasmid pBI121 with a selectable marker gene for *nptII* (neomycin phosphotransferase). Transformed cells were cultured on kanamycin (50 mg/L) and cefotaxime (300 mg/L). Proliferation of callus was achieved, with complete suppression of *Agrobacterium*. About 50-60% calli showed GUS ( $\beta$ -glucuronidase) expression, confirming transformation. Thus, genetic transformation of okra was successfully achieved by optimizing various parameters for regeneration and *Agrobacterium* infection. The regenerated plants were successfully hardened in earthen pots after adequate acclimatization.

**Leena Lavanya Draviam, Narayanaswamy Papanna (India), Luke Simon (UK/India)** Genomic Analysis of Open Pollinated Progenies of Mallika Mango (*Mangifera indica* L.) using RAPD Markers (pp 7-11)

### ABSTRACT

**Original Research Paper:** DNA-based RAPD (Random Amplification of Polymorphic DNA) markers have been used extensively to study genetic relationships in a number of fruit crops. A wide genetic diversity exists in mango fruit in India. In this study, seven commercial varieties of mango and with their corresponding open pollinated progenies of 'Mallika' (OPPM) were screened using RAPD markers with decamer primers of arbitrary sequence. Out of the 210 primers screened, 10 were selected which gave 86 clear and bright fragments. A dendrogram based on co-efficient of similarity implied a low degree of genetic diversity among the varieties and open pollinated progenies used for experimentation. The open pollinated progenies had deviated from their parents except for 'OPPM-5' and 'OPPM-13' grouping with their paternal parents. The hybrids 'Amrapali', 'Sindhu' and 'Mallika' were clustered with both their maternal and paternal parents. The dissimilarity matrix showed a maximum genetic difference of 17% between var. 'Ratna' and 'OPPM-13' genotype a progeny of 'Amrapali' and a low genetic difference of 2% between the variety 'Sindhu' and 'OPPM-5' genotypes, where 'OPPM-5' is an open pollinated progeny of 'Sindhu'. RAPD analysis proved to be a quick, simple and significant testing method to assess genetic diversity among mango populations studied.

**Rajinder Kaur, Shivani, Bhawna Saxena, Hardayal Singh Kanwar, Netar Parkash Dohroo, Sadiq Majeed, Dile Ram Sharma (India)** Detecting RAPD Markers Associated with Black Rot Resistance in Cabbage (*Brassica oleracea* var. *capitata*) (pp 12-15)

### ABSTRACT

**Original Research Paper:** A RAPD marker linked to resistance (assayed as absence of development of V-shaped lesions) to black rot (*Xanthomonas campestris* pv. *campestris*) in cabbage was identified using a mapping population of 200 F<sub>2</sub> (January King  $\times$  Golden Acre) plants with January King being the resistant parent. The analysis of genetics of black rot resistance was based on parents and the F<sub>2</sub> generation. Resistance in cabbage appeared to be governed by a single gene (monogenic). A total of 191 ten random primers were used to survey the parental polymorphism with regard to DNA amplification by polymerase chain reaction. The primers which showed polymorphism in parental lines were used for bulked segregant analysis. The primers which amplified reproducibly in the resistant and susceptible bulks were used for single plant analysis of 200 F<sub>2</sub> plants. A RAPD marker C-11<sub>1000</sub> (5'-AAAGCTGCGG-3') flanking the black rot resistance gene with a distance of 3.1 cM, was identified. This marker was close enough to the black rot resistance gene to allow a dependable marker-assisted selection (MAS) for black

rot resistance. However, more closely linked markers, if identified, would improve the effectiveness of MAS. Selection of plants on the basis of markers is more straightforward than that based on phenotype.

**Naima Osman Laajimi (Tunisia), Farhat Abbas (USA), Salah Rezgui, Mongi Zekri, Rachid Hellali (Tunisia)** Effect of Deficit Irrigation on Apricot (*Prunus armeniaca* L.) cv. 'Amor El Euch' Trees Grown in the Mediterranean Region of Tunisia (pp 16-21)

#### ABSTRACT

**Original Research Paper:** The response of apricot trees (*Prunus armeniaca* L.) to deficit irrigation (DI) was studied in two cropping seasons using a local cultivar 'Amor El Euch' in a Mediterranean region of Tunisia. The specific objectives were to evaluate the effects DI on 1) the soil-water availability in the tree root zone, 2) the plant and fruit growth parameters including leaf proline content, various fertility variables, fruit quality, and fruit yield, and 3) the water use efficiency (WUE) and water savings for mature 'Amor El Euch' apricot trees. Regulated irrigation (RI) treatments included: RI-1: irrigation to fulfill 100% crop consumptive use or evapotranspiration ( $ET_C$ ) from phenological stage I through IV (control); RI-2: irrigation to fulfill 50%  $ET_C$  from stage I through IV; RI-3: irrigation to fulfill 100%  $ET_C$  during stages I and II, and 50%  $ET_C$  in stages III and IV; and RI-4: irrigation to fulfill 100%  $ET_C$  during stages I, II, and III (preharvest) and 50%  $ET_C$  in stages III (postharvest) and IV. Results showed that soil-water content were within the readily available water (RAW) level in the 2003 cropping season and below the RAW level during the 2004 season most probably due to 50% more total rainfall received during the prior season. The DI resulted in a significant increase in the leaf proline content during both seasons probably due to the developed response of trees to drought stress. Fruit diameter, length, and degree of firmness increased with an increase in water stress. On the other hand, fruit yield was significantly lower for RI-2 (2003, 2004) and RI-3 (2004) treatments than that for the control treatment. There was no significant decrease in fruit yield for RI-3 (2003) and RI-4 (2003, 2004) compared with the control treatment. Increased WUE resulted in up to 50% irrigation water savings during the DI treatments.

**Mahmoud M. Shaaban (Egypt)** Injection Fertilization: A Full Nutritional Technique for Fruit Trees Saves 90-95% of Fertilizers and Maintains a Clean Environment (pp 22-27)

#### ABSTRACT

**Research Note:** Several experiments were carried out in Egypt and Germany to study whether fruit shrubs and trees can be fertilized by injection through the trunk. Results showed that dicotyledonous vascular trees (mango and grapevine) can be fully fertilized by injection through xylem. Only 5-10% of the levels used in soil fertilization were sufficient for good growth and high yield. Growth of the injection-fertilized mango (*Mangifera indica* var. 'Sukkary white') trees was 20-25% higher than soil-fertilized plants while in grapevine (*Vitis vinifera* vars. 'White Riesling' and 'Spaet Burgunder') fruit yield increases were 32-49% higher compared to soil fertilization. Fruit quality of grapevine clusters assessed (juice °Brix, pH, reduced sugars, total acidity, grape vinegar, apple vinegar, ethanol and glycerin content) of the plants fertilized through injection was better than those fertilized through soil. Grapevine fresh juice content of the reduced sugars and ethanol increased by 7.5-11.9 and 41.4-50%, respectively while the total acidity decreased by 6.2-19.7%. Using injection fertilization, there was no need to control weeds because they never competed with tree roots for nutrient absorption. Since there is no soil fertilization, there was no use of herbicides or pesticides, nor leaching of these compounds to underground water, which was expected to be clean enough to be used as drinking water with no or less health hazards. This technique was registered as a patent in the Egyptian Academy for Scientific Research and Technology number 23750 in July, 2007.

**Ali Bacha Nabeela, Kinan Darkazanli, Ahmad M. Abdul-Kader (Syria)** Direct Organogenesis and Plantlet Multiplication from Leaf Explants of *in Vitro*-Grown Shoots of Apple (*Malus domestica* Borkh.) cv. 'Golden Delicious' and MM111 Rootstock (pp 28-34)

#### ABSTRACT

**Original Research Paper:** The aim of the present study was to develop an efficient direct shoot formation system for apple (*Malus domestica* Borkh.) cv. 'Golden Delicious' and 'MM111' rootstock as a prerequisite for genetic transformation with antifungal genes and also as a method for rapid clonal multiplication. Adventitious shoot formation from leaf discs of 'Golden Delicious' and 'MM111' was achieved using leaves from *in vitro*-grown plants. Optimum conditions for 'direct' shoot organogenesis resulted in 92 and 90% of the explants producing one or more shoot per explant with high regeneration rate of 4 and 4.1 in 'Golden Delicious' and 'MM111', respectively on MS basal medium containing 1.0 g/l MES (morpholino ethanesulfonic acid), 2.0 mg/l TDZ, with 0.2 mg/l NAA. Organogenesis did not occur on media without cytokinins. The

organogenic capacity of leaf pieces was dependent on the leaf maturity and the origin of the leaf piece with the youngest light green expanding leaves being more regenerative than the older ones. Middle leaf segments were more responsive than the upper or lower part of the leaf. Adventitious shoots originated from both cut areas and from surfaces of the wounded leaf explants. Shoot multiplication was achieved on media consisting of MS media supplemented with B5 vitamins, 1.0 g/l MES, 30 g/l sucrose, 1 mg/l BAP, 0.3 mg/l IBA, 0.2 mg/l GA<sub>3</sub> and 6 g/l agar and were subcultured every 4 weeks. *In vitro* rooting was achieved easily by transferring 2-3 cm long shoot tips to rooting ½ MS basal medium supplemented with 1.0 mg/l indole-3-butyric acid (IBA). Multiplied plants were successfully acclimatized and cultivated in the field under natural conditions to evaluate their phenotypic uniformity and field performance.

**Reza Bahmani, Mansour Gholami, Hamid Abdollahi, Omid Karami (Iran)** The Effect of Carbon Source and Concentration on *in Vitro* Shoot Proliferation of MM.106 Apple Rootstock (pp 35-37)

#### ABSTRACT

**Research Note:** The effect of fructose, sucrose, glucose, sorbitol, and maltose at various concentrations on *in vitro* shoot proliferation on MM.106 apple rootstock was studied. Shoot tips were cultured in MS medium containing 0.4 µM indole-3-butyric acid (IBA), 4.43 µM benzylaminopurine (BAP), 0.6% agar and 0, 30, 60, 90, or 120 mM of the above carbohydrates. Type and concentration of sugars had a significant effect on shoot number and length. Sorbitol at 90 mM was the most effective carbon source for shoot proliferation and it improved plant regeneration. Shoot number was lowest at 30 and 120 mM sucrose, 30 mM glucose and at all concentrations of maltose. Shoot survival in the presence of all carbohydrates was >85% after four weeks. The reduction in shoot length and number was more pronounced in maltose compared with the other sugars.

**Dilip Nandwani (USA)** Field Trials of Tissue Culture Taro (*Colocasia esculenta* (L.) Schott) in the Northern Mariana Islands (pp 38-43)

#### ABSTRACT

**Original Research Paper:** The Commonwealth of the Northern Mariana Islands consists of a group of islands in the western Pacific. Taro (*Colocasia esculenta* (L.) Schott) is one of the important subsistence food crops grown in the Northern Marianas. The quality and production of taro crop has been severely affected in recent years by the introduction of major diseases and pests impacting taro production. The downturn in the islands' economy, natural disasters such as droughts and typhoons, and paucity of quality planting material have also had considerable constraints on taro production in the Commonwealth. The Cooperative Research, Extension and Education Service Department of the Northern Marianas College introduced a tissue culture program for the *in-vitro* multiplication and propagation of quality planting material of taro. New varieties of taro produced *in-vitro* were introduced from the regional certified institution and evaluated for tolerance to insect pests and diseases, plant growth, corm quality, high yield, taste and superior agronomic characters. Twenty-three varieties of taro propagated through tissue culture were tested at the Agriculture Experiment Station. Field trials and taste testing of new varieties were successfully conducted throughout the islands of Saipan, Rota and Tinian. Results indicated that new varieties performed well in local soil and climatic conditions of the Northern Mariana Islands. Japanese varieties (CA/Jp-01, 06 and 09) rated excellent for taste, quality of corms, easy cooking, plant growth and yield comparable to other varieties. The selected varieties of taro were identified as the economically and culturally important ones that have been tested for superior characteristics in the region.

**Haim Nerson (Israel)** Source-Sink Relationships and their Effects on Fruit Growth and Quality in Casaba Melon (*Cucumis melo* L. var. *Inodorous*) (pp 44-47)

#### ABSTRACT

**Original Research Paper:** Two field experiments were conducted in two successive spring-summer seasons at the Newe Ya'ar Research Center (northern Israel) to examine source-sink relationships in 'Noy Amid', a Casaba-type melon. The source and sink were artificially manipulated by removing leaves and fruit shortly after fruit-set. Highest fruit yield and fruit quality were obtained in control plants and any reduction in source size (by leaf removal) resulted in yield decline. In 'Noy Amid' melon, plants with 4-8 leaves could not support more than one fruit and all other young fruits aborted. The weight of mature fruits was reduced with decreasing source size (leaf number). The sink size (number of young fruits) at the beginning of treatment application had a significant effect on assimilate production and translocation to the fruits. Plants with 2 young fruits as compared to a single fruit, and with 4 young fruits as compared to 2 fruits produced larger mature fruits with the same number of leaves. These data strongly support the idea that sink size affects the photosynthetic activity of the leaves, which is increasing

by increased demand of the sink organs. Decreased source size had a negative effect not only on fruit yield but also on fruit quality characters. Fruit shape, flesh perfection and total soluble solid content were all adversely affected by artificially reducing leaf number.

**Javier Hernán Pereira da Costa, Gustavo Rubén Rodríguez, Guillermo Raúl Pratta, Roxana Zorzoli, Liliana Amelia Picardi (Argentina)** Characterisation of Tomato Germplasm by Pericarp Protein Profiles and Morphologic and Biochemical Fruit Traits (pp 48-53)

#### ABSTRACT

**Original Research Paper:** A diversity of genetic materials contained in traditional varieties, modern cultivars and wild species contributes to plant genetic resources. The aim of this work was to characterise different tomato genotypes by pericarp protein profiles and morphologic and biochemical fruit traits. Highly significant differences were found between these genotypes for all traits and the cluster analysis allowed to separate cultivated genotypes from the others. Pericarp protein profiles were obtained at two ripening stages to classified these genotypes. These protein profiles obtained at mature green and red ripe stages showed high degree of polymorphism. Genotype clustering by pericarp protein profile was different to that obtained by morphological and biochemical fruit traits.

**Hui-lian Xu (Japan), Feifei Qin (Japan/China), Laurent Gauthier, André Gosselin (Canada)** Greenhouse Tomato Growth and Physiological Responses to High Nutrient Solution Electrical Conductivity and Low Substrate Water Content (pp 54-61)

#### ABSTRACT

**Original Research Paper:** The ionic concentration of a nutrient solution, shown by its electrical conductivity (EC), has profound effects on tomato plant growth and fruit yield in the greenhouse. However, high EC effects cannot be simply attributed to restricted water uptake by rhizosphere salinity as usual. To understand the differences in effects of high EC and substrate water deficit, tomato plants were grown in peat-moss based substrate with a nutrient solution of high ( $4.5 \text{ dS m}^{-1}$ ) or low ( $2.3 \text{ dS m}^{-1}$ ) EC under high (95% of capillary capacity) and low (55%) substrate water content (SWC), and examined were the effects on growth, yield, photosynthesis, and plant water relations. Salts were intentionally allowed to accumulate in the substrate for seven weeks by placing the pot in a dish without leaching. Both high EC and low SWC significantly decreased plant growth, dry matter production and fruit yield as well as photosynthesis, leaf water and turgor potentials, stomatal conductance and transpiration. However, blossom-end rot of fruit was more severe in high EC than in low SWC although the leaf Ca content was similar in these two stress treatments. Moreover, soluble protein content and Rubisco activities on a leaf area basis were not decreased by high EC but decreased by low SWC. Results suggested that high EC was different from substrate water deficit in effects on some physiological processes. Further research is needed to elucidate the detailed mechanism of high EC effects.

**Ibisime Etela (Nigeria/Canada), Solomon Olufemi Afuape (Nigeria), Jitendra Paliwal (Canada), Ebenezer Chukwuemeka Nwauzor (Nigeria)** Screening Sweetpotato Germplasm for Starch, Flour and Feed Quality Characteristics (pp 62-67)

#### ABSTRACT

**Original Research Paper:** Studies were conducted to screen newly collected sweetpotato germplasm for starch, flour, and feed quality traits. Fresh tuber and forage yields were evaluated in the first study using 13 genotypes while in the second study tuber dry matter (DM) and starch contents were investigated using 17 genotypes both in augmented incomplete block designs. In the third study, voluntary dry matter intake (VDMI) was determined in White Fulani cows fed with sole fresh sweetpotato forage in early lactation, and potential degradation trial in three rumen-fistulated N'Dama steers using the *in-sacco* technique in a completely randomized design. Mean ( $\pm$ SE) fresh tuber yield ( $13.00 \pm 0.471 \text{ t/ha}$ ) was higher ( $P < 0.01$ ) in control than test genotypes ( $13.20$  versus  $12.94 \text{ t/ha}$ ) while, flour yield ( $35.71 \pm 1.351$ ) was lower in control than test genotypes ( $34.80$  versus  $38.74 \text{ g/100 g}$ ). Tuber DM ranged ( $P < 0.01$ ) from  $44.27$  to  $67.11 \text{ g/100 g}$ , and starch content from  $19.80$  to  $30.60 \text{ g/100 g}$ , with a negative correlation ( $r = -0.65$ ;  $P = 0.05$ ) observed between both parameters. The orange- and white-fleshed genotypes were similar ( $P > 0.05$ ) in the agronomic parameters determined. Forage crude protein (CP) ranged ( $P < 0.05$ ) from  $116.1$  to  $221.0 \text{ g/kg DM}$ , neutral detergent fibre (NDF) from  $376.4$  to  $482.0 \text{ g/kg DM}$ , and  $\beta$ -carotene from  $320$  to  $460 \mu\text{g/100 g}$ . Voluntary DMI, percent of liveweight, and potential degradability of DM varied ( $P < 0.05$ ) from  $7.4$  to  $9.0 \text{ kg/d}$ ,  $2.9$  to  $3.7\%$ , and  $837$  to  $885 \text{ g/kg DM}$ , respectively. The positive correlation ( $r = 0.92$ ;  $P = 0.05$ ) between flour yield and forage NDF suggests the possibility of developing models for predicting forage quality based on both agronomic and nutritional data.

**Gamal Riad, Abdalla Ghoname, Ahmad Ahmed, Mahmoud Abd El-Baky, Amira Hegazi (Egypt)** Cabbage Nutritional Quality as Influenced by Planting Density and Nitrogen Fertilization (pp 68-74)

#### **ABSTRACT**

**Original Research Paper:** A field experiment was conducted to investigate the effect of plant density and nitrogen fertilization levels on the growth, yield and nutritional quality of cabbage (*Brassica oleracea* L. var. *capitata*), during the two winter seasons of 2006 and 2007. Four rates of nitrogen fertilizer 0 (control), 30, 60, or 90 Kg N/feddan (1 feddan (fed) = 4200 m<sup>2</sup>) were combined with three plant densities (2, 3, or 4 plants/drip) which resulted in a density of 4, 6, or 8 plants/m<sup>2</sup>. Data showed that increasing both N fertilization rate and planting densities significantly increased plant height while head diameter, length, weight, edible head weight, and compactness rate were increased only with increasing N fertilization rate. However, a negative impact of the highest planting density on all these parameters was recorded. Total yield/fed significantly increased by increasing both N rate and planting density. The highest yield was obtained by the application of 90 kg N/fed combined with 8 plants/m<sup>2</sup>. Increasing N fertilization rate increased total soluble solids (TSS) but decreased dry matter content; meanwhile, planting density did not significantly affect both of these parameters. Nitrogen, protein, and nitrate contents generally increased with increasing N fertilization rate but decreased with increasing planting density. Antioxidant capacity expressed as total phenols and vitamin C contents was positively affected by increasing N fertilization rate although it was not significantly affected by increasing planting density.

**Girija Ganeshan, A. Manoj Kumar, Krishna Reddy, R. Saraswathi (India)** Molecular Identity of *Fusarium oxysporum* Isolates Collected from Karnataka State (pp 75-78)

#### **ABSTRACT**

**Original Research Paper:** *Fusarium* species cause wilt, yellowing and root rot in most horticultural crops. *Fusarium oxysporum* generally causes wilt and has many *formae specialis* depending on the host. To know their exact identity, the present study was carried out to differentiate isolates collected from different agro-climatic regions of Karnataka State using molecular methods. *Fusarium* species affecting onion, musk melon, tomato, okra and pea were isolated from infected plants, purified and then multiplied on potato dextrose broth. Isolated DNA of 20-d cultures was amplified by PCR using primers specific to the ITS region. Out of 5 cultures, 4 isolates belonged to one group. The 4 isolates showing the same bands were further differentiated by RFLP analysis using *AluI* to confirm whether they belonged to the same *formae specialis*. Based on PCR-RFLP analysis of amplified product of On-5 and To-3, both isolates were found to be the same; similarly, Mu-2 and Ok-1 were the same while other isolates were different.