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#### **ORAL PRESENTATIONS**

## S1-KEYNOTE 1

Advances of genome sequencing and its applications in Chinese jujube (Ziziphus jujuba Mill.)

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Genome-sequencing is the key to crack genetic code and is of significant strategic value to the molecular improvement of plants. Chinese jujube (Ziziphus jujuba Mill.), a major dry fruit and a traditional Chinese herbal medicine, is the most valued member of family Rhamnaceae. We carried out the de-novo genome-sequencing of Chinese jujube in 2010, and published the first whole genome sequence of jujube (Z. jujuba Mill. 'Dongzao') and Rhamnaceae in 2014. Two years later, the second genome sequence of jujube (Z. jujuba Mill. 'Junzao') was reported by another research team. The previous researches showed that the genome of Chinese jujube is relatively small (360-440Mb), but is of high complex in terms of high heterozygosity (1.9%), densely and evenly distributed SSR (378.1/Mb) as well as low GC content (33.41%). A total of 32,808 protein-encoding genes comprising 3,084 jujube-specific genes were predicted, of which 23,996 ones were anchored to the 12 pseudochromosomes constructed by the aid of a high density SNP genetic map. The jujube genome underwent frequent inter-chromosome fusions and segmental duplications but no recent whole-genome duplication. Population structure analysis revealed the complex genetic background of jujube resulting from extensive hybridizations between Chinese jujube and its wild relative. Comprehensive phylogenetic analysis basing on genome sequences supported the transfer of family Rhamnaceae from order Rhamnales into Rosales. Comparative genome analysis coupled with transcriptome and re-sequencing data revealed the molecular mechanisms underlining some of the jujube's distinct properties including extreme accumulation of ascorbic acid (vitamin C) and sugar in fruit, selfpruning shoot system and outstanding tolerance to biotic and abiotic stresses. A number of key genes involved in fruit organic acid and sugar metabolism and S-locus genes controlling gametophytic self-incompatibility were identified. In addition, genome-wide screening of SSR markers, reference genes and functional genes, and large scale re-sequencing and genome wide association study (GWAS) have been carried out in jujube. A database of jujube genome was also constructed and open to the public. In the near future, multi-omics analysis and genomeassisted breeding will greatly promote both the basic and applied research in jujube.

Keywords: Jujube, genome sequencing, multi-omics, application, research advances

## S1-KEYNOTE 2

#### Marketing and Economics of Ber Production in India

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Success of ber grower considered fruitful when his produce sell at reasonable good price in the market and cultivation proved profitable. The process of ber marketing involves many constraints like cost of transportation, packaging, loading and unloading of fruits. Ber harvesting coincides with harvest of wheat and other Rabi crops. Growers have to deal with various marketing hurdles and functionaries like commission agent and wholesaler. This all is a cumbersome process and involves some uncertainties in smooth marketing of ber. The growers generally auction their ber crop to the contractors. They harvest the fruits according to their convenience and sell it in local or distant markets. Grafted ber bear 100-200kg fruits per tree depending upon the cultural practices followed. Large (A grade) and medium (B grade) sized fruits comprises 60 per cent of total yield of a tree. Ber fruits are preferably packed in CFB box of 4 kg capacity to get higher premium in the market. Small sized and



culled type fruits are packed in baskets or gunny bags. Ber fruits of A and B grades fetch higher price of Rs 15-20/kg but small sized C grade fruits earn Rs 8-10 per kg in the market. Economics of ber cultivation is comparable with other fruit crops and ber growers can net an income between Rs 1.5-2.0 lakhs/ha. Closer spacing at 6 x 6 m can be made on semi- vigorous rootstock. Double numbers of plants are accommodated per unit area and 20 per cent higher yield can be obtained. Ber fruits are generally sold in local markets but demand for export is growing. During 2016-17, 1074 mt fresh ber fruits with value of Rs 5.38 crores were exported from India.

Keywords: Transportation, Packaging, Contractor, CFB boxes, Closer spacing, Economics, Export

#### S1-P1:

Some critical issues in jujube production in the United States

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Jujube cultivars were first imported into the U.S. from 1908-1918. As more consumers are becoming concerned about jujube fruit nutritional value, growers are interested in jujubes for its reliable crop and drought tolerance. With the severe drought in the Southwest in recent years, more growers are interested in planting jujubes. In general, jujubes grow and produce well in the U.S. especially the Southwest. To promote jujube production to an industry in the U.S., more detailed work is needed. Critical issues face the jujube industry in the U.S.: 1) Research and extension support: Jujube research and extension activities, and publications, are limited in the U.S. Most jujube related publications are not in English. Growers are frustrated and cannot get the necessary information. 2) Cultivar recommendation/selection. Researchers are responsible for recommending cultivars for different end uses. 3) Plant availability. A waiting period of 1-2 years is not uncommon for jujube plants from dominant jujube nurseries in the U.S. Growers cannot get the cultivar they prefer and neither can they get the plants on time. Jujube production represents an untapped market in the nursery industry and techniques such as tissue culture could ramp up production and ensure plant availability in the U.S. 4) Jujube cultural management. Research into jujube training systems/pruning techniques, water requirement, and fertility management are lacking. Jujube fruits are harvested manually in the U.S. Mechanical harvest, especially for drying cultivars, could reduce labor cost. 5) Jujube fruit postharvest treatment, cold storage, processing and value-added product development. Researchers can identify suitable cultivars for cold storage and processing, and develop several dominant products and necessary machinery/equipment. 6) Jujube marketing and promotion which includes the fresh/dry fruit and processed products. Promoting jujubes will make more consumers aware of this nutritious fruit and increase demand.

Keywords: Ziziphus jujuba, cultivar, plant availability, culture management, processing, marketing

# S1-P2:

Advances and Achievements on Chinese Jujube Breeding in China

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Chinese jujube (Ziziphus jujuba Mill.), an important fruit tree species native to China, has a cultivation and utilization history of more than 7000 years. It is distributed throughout China now with a very high diversity of germplasms which provides a wide background for breeding. In this paper, the breeding objectives, techniques and achievements in Chinese jujube were reviewed and summarized. The breeding objectives on Chinese jujube changed with the development of economy and the improvement of people's living standards from focusing on high yield and big fruit to good quality, high nutrition, easy-management and high resistance to fruit shrinking and cracking. Nowadays, Chinese jujube breeding practice includes systematic selection, cross breeding, polyploid breeding and transgenic breeding. Selection from bud mutation, local rare germplasms and seedlings is still a main way to obtain new cultivars in Chinese jujube. Cross breeding of Chinese jujube is very difficult owning to its small flower, cleistogamy phenomenon as well as high rate of embryo abortion. In view of the



above obstacles limiting cross breeding, we first carried out researches on special germplasms screening. We found a number of germplasms suitable for parents, such as male sterile, self-fruitless or self-sterile germplasms, and those rich in cylic nucleotides, saponins, flavones, triterpenic acids, water soluble polysaccharides, Vc, or resistant to fruit cracking or shrinking etc. Thereafter, techniques of controlled hybridization, embryo rescue and maker assistant selection were explored to increase the hybridization efficiency and improved in cross breeding. Polyploid breeding were even better practiced and a number of pure polyploids were acquired by combining colchicine application with in vivo bud regeneration technique. An obvious progress has been made in transgenic breeding recently. Some excellent cultivars for table and dehydration were also briefly induced in the paper.

Keywords: Chinese jujube, Zizhiphus, breeding, objectives, techniques, cultivars

#### S1-P3:

## Jujube(Ziziphus jujuba) in Republic of Korea: Major Cultivars and Prospect of Jujube cultivation

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The Jujube(Ziziphus jujuba Mill) cultivation was recorded at 900 years ago(1,100, Goryeo Dynasty) in Korean Peninsula. It is assumed that Jujube had been introduced into Korea from China. The Jujube cultivation area was dramatically decreased by Jujube Witches-broom Phytoplasma in 1970s. Recently, Jujube was planted in 3,000ha(7,000farmer) and its production was 14,000ton(\$80million) in Korea. The main Jujube cultivation area are Gyeong-San(1400farmer, 840ha, 4,300ton), Cheongdo(2650farmer, 580ha, 4,200ton), Gunwi(930farmer, 460ha, 2,300ton), Boeun(1400farmer, 700ha, 2,500ton) and Miryang(590farmer, 370ha, 1,500ton). Nowadays, the pattern of Jujube consumption is changed Dried Jujube into Fresh Jujube. Also the cultivation method is changing. The most important popularly cultivated cultivars in Korea are 'Bokjo', 'Boeun', 'Geumseong', 'Wolchul' and 'Mudeung'. The average of five Jujube cultivar fruit size, weight and Brix degree were observed in 3.1-3.6cm×2.4-2.6cm(length×width), 10.8-12.7g and 31.4-37.1Brix, respectively. Consumption of fresh Jujube is expected to be increase in the future, so that cultivation for fresh Jujube will be increase.

Keywords: Jujube, Ziziphus jujuba, Jujube cultivar, Korean Jujube, Jujube cltivation

# S1-P4:

#### Crop Regulation Practices for Ber (Indian Jujube) in India

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Adoption of crop regulation practices becomes necessary for economic and commercial ber production. Bulk of immature fruits dropped during initial stage of growth and development due to hormonal imbalance, abortion of embryo, incidence of diseases and inclement weather. Finally one-fourth of total crop attained marketable size (25-30g). Ber cracking is common in Sanaur-2, ZG-2, Kaithli and Gola varieties due to their soft texture skin and high TSS at maturity. Rise in temperature in March, endogenous level of growth hormones, irregular irrigation interval and varietal characters contributes in cracking. NAA spray at 20-30ppm in October and November check physiological fruit drop and reduces fruit cracking. Watering to ber trees at proper interval helps in regulating water uptake and solutes and minimized fruit cracking. Ber stops in growth, shriveled and falls due to severe cold and cold waves during December-January in north India. Light irrigation and burning dried leaves slowly can save to some extent the heavy drop occurred during winter months. Foliar application of potassium nitrate 0.5% produced better sized fruits and high yield. White ants attack root system, main stem and main limbs and block smooth flow of sap in xylem and phloem which results into fruit drop. Treatment of tree basins with chlorpyriphos @1ml/litre of water in April and September and paint with its thick solution (100ml/litre water) to trunk and main limbs results in drop reduction. Fruit drop due to fungus attack can be checked by spraying bayleton 0.05% at monthly interval from October to December. Late cultivar Umran do not ripen uniformly and picked in 4-5 lots. Ethephon 400-500ppm at colour break stage advances ripening by two weeks and produce attractive, uniform and better quality ber.



Keywords: Fruit drop, Fruit cracking, Cold, White ant, Potassium nitrate, Umran Ethephon

## **S2-P1:**

# Preliminary Results about Romanian Consumers Preferences on Jujube Fruits

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First jujube trees (Ziziphus jujuba) have been introduced to Romanian lands probably by the Greek and Byzantine colonists nearly 2000 years ago. Local biotypes of sour jujube (Ziziphus acidojujuba) have been founded recently in Jurilovca on Razelm Lake shore and a genotype of Z. jujuba, in Ostrov on Danube shore. After 1996, around 20 jujube varieties and local populations were introduced from China and started to be studied in the Romanian conditions at the Faculty of Horticulture experimental fields in Bucuresti. In order to study the consumer preferences regarding the jujube fruits, several sensorial analysis session were organized, both for fresh and dried fruits. Fruit size, color, taste, flavor and overall impression were analyzed by consumers of different ages and gender. In parallel, some fruit physical and chemical analysis were made to characterize the fruit samples. The results showed that the consumers preferences are influenced by age and gender and the jujube fruits are highly appreciated, both fresh and dried. Further consumer tests are needed to better understand their preferences and the typology of new jujube genotypes, adapted to the local growing conditions that will be extended in production.

Keywords: Ziziphus jujuba, sensorial analysis, age, gender

#### S2-P2:

Selection and Multiplication of an Elite Genotype of Ziziphus spinachrisri from Seedling Populations in Kuwait

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Different species of Ziziphus such as Z. spinachristi, Z. mauritiana, Z. xylopyrus and Z. jujuba were introduced in Kuwait due to its ability in tolerating the harsh arid environmental climatic conditions. Due to the pollen compatibility between different species of Ziphus, several superior genotypes were noticed in the seedling populations. Recently, a project study was carried out in the Kuwait Institute for Scientific Research (KISR) on genotype selection from seedling populations of Z. spinachristi for the nutritive fruits. Seeds were collected from the locally grown Z. spinachristi and 5,000 seedlings were produced. Among the 5,000 seedlings, 500 plants were planted in the field. After one year of planting, all the plants started producing flowers and fruits. Most of the plants were varied in their fruit quality (size, shape and taste). A stone-less superior genotype was identified during the field evaluation. The selected genotype has produced sweet stone-less large fruits with attractive color. The fruits have fertile seeds without a hard stone around. Two viable seeds without stone were observed in each fruit until the fruit maturity and the seeds aborted during the ripening stage due to the lack of stone around the seeds. Tissue culture technology was applied for the mass clonal propagation of this elite genotype. A technique for rapid in vitro multiplication, rooting and establishment of plantlets in the field was developed and described. The influence of growth regulators in the regeneration, growth and multiplication medium on plantlet growth and rooting was assessed. Rapid propagation was achieved by the zygotic embryo rescue, axillary shoot multiplication and somatic embryogenesis methods. Plants were established successfully in the greenhouse through the maintenance of a high humidity environment for 30 days and hardened plantlets were subsequently transferred to the field. Field evaluation of these micropropagated plants confirmed the clonal nature through producing true-to-type fruits similar to the mother.

Keywords: Ziziphus spinachristi, stone-less fruits, clonal micropropagation and field evaluation.



#### S2-P3:

# A Review of the anticancer and antioxidant components of Ziziphus jujuba Mill. (Jujube) Fruits

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Ziziphus jujuba Mill (Jujube) is a fruit-bearing plant belonging to the Rhamnaceae familyand the Ziziphus genus. The fruit not only has a food function with high nutritional, but also has a potential medical and health care function especially its anticancer and antioxidant activity. Current studies show that the main anticancer and antioxidant active components of jujube fruits contain flavonoids, polysaccharides, terpenoids, cyclic adenosine monophosphate, phenolic acids, etc. In this review, we aim to summarize the reported activity extract in jujube fruit, the demonstrated extraction, separation, purification, and determination procedure related to these anticancer and antioxidant extracts, which can be further developed as leading compounds for applied in health food and pharmaceutical products.

Keywords: Jujube; anticancer; antioxidant; active components

## S2-P4:

In vitro immunological and anti-complementary activities of two water-soluble lignins from Zizyphus jujube cv. Jinchangzao

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Two homogenous biological macromolecules, designated as JJC1 and JJC2 were extracted from Zizyphus jujube cv. Jinchangzao. Their molecular weights were determined to be 56.03 and 112.11 kDa by high performance gel permeation chromatography (HPGPC), respectively. Chemical and spectral analysis indicated that both JJC1 and JJC2 were predominant in lignin, along with ~18% carbohydrates. We examined whether lignin could stimulate the proliferation of spleen lymphocytes and enhance phagocytosis and NO production of RAW264.7 cell. Lignins were evaluated in anti-complementary activity and showed JJC2 was inhibitory effect on complement activation through the classical pathway (CH50: 2.73 mg/mL) and the alternative pathway (AP50: 2.99 mg/mL), but JJC1 did not have such effects. These finding implied that water-soluble lignins derivatives were one of bioactive components in Zizyphus jujube, and further provided insights into the understanding of molecular basis for diverse medicinal and nutritional values of this jujube.

Keywords: Anti-complementary activity; immunological activity; jujube lignin.

#### S2-P5:

# Optimization of a High-Density Genetic Map for Chinese Jujube and QTL Mapping for freezing tolerance

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Chinese jujube (Ziziphus jujuba Mill, 2n=2x=24), belongs to the Rhamnaceae, which is an economically important Chinese fruit tree. Jujube production occupies an important position in China's agriculture. Improved varieties are the basis for the healthy development of jujube production. Conventional genetic strategies, up to now, have been employed to jujube improvement work but with limited progress, such as laborious and time-consuming. Marker assisted selection (MAS) has been proven to be a promising solution for quickening breeding of fruit trees, but jujube lags behind other fruit crops in molecular genetic map construction and marker



assisted selection. In this study, 99 F1 progenies derived from the cross 'Dongzao' × 'Yingshanhong' were used as mapping population. We detected SNP and InDel by restriction-site-associated DNA sequencing (RAD-seq) and alignment of short reads on a reference genome. Subsequently, we constructed a high-density linkage map of jujube using SNPs and InDels integrated with SSRs the laboratory has acquired. The quantitative trait loci (QTLs) for freezing Tolerance were detected in the integrated map. Illumina sequencing of reducedrepresentation libraries generated a total of ~98.76Gb clean reads from parents and 99 F1 progenies. A total number of 7939 markers (7026 SNPs and 913 InDels) that follow Mendel's segregation ratio were identified through mapping the RAD reads to the reference genome of 'Dongzao'. Genetic map was constructed based on pseudo-testcross theory combine with the SSR markers. Finally, the linkage map consists of 4173 SNP markers, 486 InDel markers and 46 SSRs, 4669 markers in total, spanning 2643.79 cM, with an average marker distance of 0.57 cM. The integrated map contained 12 linkage groups (LGs), consistent with the haploid chromosome number of the two parents. Of the 4669 RAD markers, 3919 were linked to the female genetic map, 4184 to the male map. Based on this high-density integrated jujube linkage map, QTL for freezing tolerance mainly distributed on LG7 were identified and positioned on the genetic map by Kruskal-Wallis model method of MapQTL 6.0 software. The results of this study are expected to provide important theoretical references for QTL detection, map-base gene cloning and molecular-assisted selection breeding.

Keywords: Jujube; RAD-seq; SNP; InDel; Gentic map; QTL

# **S2-P6:**

# Development and Evaluation of a Novel Set of EST-SSR Markers Based on Transcriptome Sequences of India jujube

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Ziziphus mauritiana, known as India jujube, is native to a wide tropical region from Vietnam, India to Sahel, and domesticated in India and has been introduced to Taiwan, South China and North Africa. India jujube produce tasty and multi-nutritional fruits, and providing a farm income in the rural region. There were rich germplasm and many elite cultivars were domesticated. The objective of this study is to develop EST-SSR markers for Ziziphus mauritiana Lam. based on the transcriptomic data and provide more molecular markers for cultivars identification, genetic diversity, fingerprint construction. We retrieved transcriptome sequences of Z. mauritiana from online database, and selected 80 Expressed Sequence Tags (EST) that containing di-nucleotide repeats for further polymorphism determination on 20 cultivars. Among which, 39 loci were successfully amplified the targeted fragments and 16 loci appeared to be polymorphism. In total, 77 alleles were checked, the number of allele for each locus varied from 2 to 12, with an average of 4.81. The polymorphism information content (PIC) values varied from 0.085 to 0.682. Those SSR markers provide a tool for studying cultivar protection, genetic map, and population structure.

Keywords: Ziziphus mauritiana, EST-SSR, molecular identification, cultivar protection

# S3-P1:

# Metabolism of fruit cell wall materials and related gene expression during jujube fruit ripening and softening

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This study explored the dynamic change of cell wall materials and related enzymes as well as the regulation mechanism of related genes in Ziziphus jujuba Mill. during fruit ripening and softening. The fruits of 'Junzao' were chosen in seven developmental ripening stages. Fruit firmness, the water content, the total soluble solid (TSS) content, the color parameters, the contents of two kinds of cell wall materials including pectin and cellulose, and the activities of three related enzymes including polygalacturonases (PG), pectinesterases (PE) and Cx-cellulase (Cx) were determined. Fifteen genes involved in cell wall metabolism were selected for analyze



their relative expression levels at different stages by qRT-PCR. With fruit ripening, the change of firmness was not significant until fruit softened, and the water content decrease. The TSS increased linearly, and the color parameters tended to be stable during three ripe stages. The pectin and cellulose contents did not change significantly but increased to an extent during ripe stages. The PG activity were undulate, while the activities of PE and Cx-cellulase increased from early ripe stage. The expressions of three PG genes decreased to very low levels. The expression levels of three PE genes reached peaks during crispy mature stages. And the expression changes of seven genes involved in cellulose and Cx-cellulase were different, in which the expression level of  $\beta$ -Glu40 were higher than others from half-red to full red stage. The content changes of cell wall materials were not significant in 'Junzao' jujube during fruit ripening and softening. In conclusion, PE genes should play a role in ripening start-up before fruit softening, and the genes involved in cellulose and Cx-cellulase regulated fruit ripening together.

Keywords: Ziziphus jujuba Mill.; ripening; softening; cell wall

#### S3-P2:

#### Strategic Management of Insect Pests in Ber (Indian Jujube): In Retrospect and Prospect

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Out of a total of about 180insect and non-insect pests feeding on ber, nearly 130 species have been recorded from Indian jujube, Ziziphus mauritiana Lamk. grown in various countries of South Asia. Out of these, the major insect pests are: three species of fruit flies (Carpomyia vesuviana Costa, Bactrocera zonata (Saunders) and Bactrocera correctus (Bezzi); leaf eating caterpillars (Euproctis spp); lac insect (Kerria spp); fruit borer, Meridarchis scyrodes Meyrick; ber beetle, Adoretus pallens and A. nitidus. Severe damage by certain species of termites and butterflies has also been noticed in certain situations. Fruit flies demand an integrated approach, to manage it. Clean cultivation / sanitation of orchards needs to be ensured by picking and destroying the infested fruits. Further to escape egg laying on fruits, harvesting at 'green and firm' stage is recommended and fruits should not be allowed to ripe on the tree. Pupae should be exposed to heat and natural enemies by raking the soil around trees during summer. Apart from insecticidal spray (500 ml of dimethoate 30EC in 300 litres of water) before harvesting, implementation of Bait Application Technique (100 ml malathion + 1 kg sugar in 100 litres of water), followed by Male Annihilation Technique, is suggested. For leaf eating caterpillars, early and small scale plucking of the leaves with egg masses and young caterpillars and their subsequent destruction is advised. Lac insect can be better managed by pruning and destruction of the infested twigs followed by the insecticidal (dimethoate) treatment. Exploitation of parasitoids and predators and exploring potential of insect growth regulators (with unique mode of action) constitute future strategies for management of the insect pests in Indian Jujube. Adoption of the suggested pest management strategies will ensure better plant health and higher economic returns.

Keywords: Insect- pests, Ber, Fruit flies, Caterpillars, Lac insect, Insecticides, Growth regulators

# S3-P3:

A novel, rapid real-time PCR detection of phytoplasma quantification and proliferation activity

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Thymidylate kinase (tmkZ) gene was isolated from Jujube Witches' Broom disease (JWB) phytoplasma. A quantitative real-time polymerase chain reaction (q-PCR) assay method were developed to detect relative content of tmkZ to jujube house-keeping gene ZjH3, the ratio of tmkZ to ZjH3 represent quantification and proliferation activity of phytoplasma at DNA and RNA level, respectively. We found that the relative quantification and proliferation activity of phytoplasma have a close relationship with JWB diseased degree. The relative

quantification of phytoplasma in JWB resistance jujube cultivar was evidently lower compare to that in sensitive cultivar after grafting on diseased rootstocks. The proliferation activity of JWB phytoplasma in sensitive jujube



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cultivar increased from 40d to 95d and decreased at 115d after grafting. However, proliferation activity could not be detected in resistance jujube cultivar.

Keywords: tmkZ; ZjH3; Chinese jujube; phytoplasma; qPCR; quantification; proliferation activity

#### S3-P4:

Characterization of Jujube (Ziziphus mauritiana L.) germplasm of arid zones of Bahawalpur based on various plant and fruit attributes

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Germplasm characterization and estimation of morphological and molecular genetic diversity provide basic information in cultivar improvement progam. Jujube or ber is an important fruit crop of arid and desert zones of the world, consisting more than 100 species of deciduous or evergreen plants, widely growing in the deserts, tropical and subtropical areas. A few local and exotic genotypes are cultivated for production of jujube fruit, enriched with various minerals, vitamins and nutrients addressing malnutrition issues of desert people. Wet it is neglected fruit crop of the country with little assessment of morphological characters. There were 18 accessions of jujube growing in arid zone of Bahawalpur, selected for characterization of plant, fruit and seed for various physico-morphological traits. Results of this study proved that all selected genotypes had broad genetic base for various commercial traits. Plants of all genotypes had significant variation regarding spread of tree canopy, stem dimensions, leaf area and its chlorophyll contents. Significantly high variation recorded regarding various fruit characters like fruit weight, fruit length, seed weight, seed length, seed diameter, fruit pulp weight and total soluble solids (TSS). Highest variation (7.02%) was recorded in fruit weight which ranged from 9.08 to 42.64 g among the tested genotypes; however, least variation was recorded in seed weight.

Keywords: Ziziphus mauritiana L; Plant characters; fruit traits; arid zone; Bahawalpur; Pakistan

