

Candidate genes associated with red colour formation revealed by comparative genomic variant analysis of red- and green-skinned fruits of Japanese apricot (*Prunus mume*)

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ABSTRACT

The red-skinned fruit of Japanese apricot (*Prunus mume* Sieb. et Zucc) appeals to customers due to its eye-catching pigmentation, while the mechanism related to its colour formation is still unclear. In this study, genome re-sequencing of six Japanese apricot cultivars was carried out with approximately 92.2 Gb of clean bases using next-generation sequencing. A total of 32,004 unigenes were assembled with an average of 83.1% coverage rate relative to reference genome. A wide range of genetic variation was detected, including 7,387,057 single nucleotide polymorphisms, 456,222 insertions or deletions and 129,061 structural variations in all genomes. Comparative sequencing data revealed that 13 candidate genes were involved in biosynthesis of anthocyanin. Significantly higher expression patterns were observed in genes encoding three anthocyanin synthesis structural genes (*4CL*, *F3H* and *UFGT*), five transcription factors (MYB-bHLH-WD40 complexes and NAC) and five anthocyanin accumulation related genes (*GST1*, *RT1*, *UGT85A2*, *ABC* and *MATE* transporters) in red-skinned than in green-skinned Japanese apricots using reverse transcription-quantitative polymerase chain reaction. Eight main kinds of anthocyanin s were detected by UPLC/MS, and cyanidin 3-glucoside was identified as the major anthocyanin (124.2 mg/kg) in red-skinned cultivars. The activity of UDP-glucose flavonoid-3- O-glycosyltransferase enzyme determined by UPLC was significantly higher in all red-skinned cultivars, suggesting that it is the potential vital regulatory gene for biosynthesis of anthocyanin in Japanese apricot.

Subjects Genomics, Molecular Biology, Plant Science

Keywords *Prunus mume*, Sequencing, Anthocyanin, UFGT, Gene expression

INTRODUCTION

Japanese apricot (*Prunus mume* Sieb. et Zucc), an attractive fruit tree, originated from Southwest China and has been extensively cultivated in all of East Asia and Japan. China, being the origin of Japanese apricot, is rich in good quality germplasm with an approximately 190 fruiting cultivars (Chu, 1999). The fruit is usually processed into many

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Research paper

Integrating proteomic and transcriptomic analyses of loquat (*Eriobotrya japonica* Lindl.) in response to cold stress[☆]Xiaoming Lou^{a,b}, Huakun Wang^{c,d}, Xiaopeng Ni^a, Zhihong Gao^{a,*}, Shahid Iqbal^a^a College of Horticulture, Nanjing Agricultural University, No. 1 Weigang, Nanjing 210095, PR China^b Suzhou Polytechnic Institute of Agriculture, No. 279 Xiyuan Road, Suzhou 215008, PR China^c Extension Center for Evergreen Fruit Tree of Jiangsu Taihu, No. 4 Xijiang Road, Suzhou 215107, PR China^d The Jiangsu Provincial Platform for Conservation and Utilization of Agricultural Germplasm, Nanjing 210014, Jiangsu, PR China

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ABSTRACT

The expression levels of many genes and the related proteins change and regulate physiological and metabolic processes that help the plant survive harsh environmental conditions under cold stress. Damage due to cold and freezing conditions often causes dynamic loss of loquat fruits in cultivated parts of northern China. To illustrate the mechanism of cold tolerance in the loquat, we combined the transcriptomic analysis with isobaric tags for relative and absolute quantification (iTRAQ) and RNA sequencing (RNA-Seq) data from loquat leaves under 4 °C treatment. The results showed 122,081 genes and 1210 differentially expressed genes (DEGs), while only 4582 proteins and 300 differential proteins (DEPs) were identified. Functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that metabolic pathways and biosynthesis of secondary metabolites were the two most common pathways in transcriptional and translational processes in this study. Comparison analysis of the transcriptomic and proteomic profiles, only 27 of 3620 genes were found to be shared both in DEGs and DEPs. Further validation with Real-Time Quantitative RT-PCR analysis showed that the genes expression of NADP-dependent D-sorbitol-6-phosphate dehydrogenase, anthocyanin synthase and phenylalanine ammonia-lyase were consistent with the pattern of transcriptome profile, which suggested that these three genes might play vital roles in cold tolerance in loquat.

1. Introduction

The loquat (*Eriobotrya japonica* Lindl.) originated in the Dadu River Valley region of south-western China. It has been cultivated over 2000 years and is now grown in more than 30 countries around the world (Chalak et al., 2014). It is an important subtropical fruit tree with great commercial value and excellent ornamental traits with evergreen leaves and a three-month bloom period from late autumn to early winter, which is also popularly employed as a garden tree.

As a commercial fruit tree, the loquat can be cultivated in maritime climates between 20°–35° north and 20°–35° south, but it can be grown up to 45° as an ornamental tree. Since the loquat tree is more tolerant to

cold stress and can blossom in winter compared to other subtropical fruit trees, it has a limited commercial production region (Lin et al., 1999). To enlarge the cultivation area, some novel germplasm and cultivars with enhanced cold tolerance have been achieved. As a result, it is more important to discover and illustrate the mechanism of cold acclimation in loquat.

Low temperature is a fatal abiotic stress, which determines the geographical distribution and use of plants (Knight and Knight, 2012). Cold stress at temperatures above zero (0–10 °C) and freezing stress at subzero temperatures are the two main types of abiotic stress. Freezing tolerance increases under cold stress, a phenomenon known as cold acclimation. Membrane structure and composition, metabolic rates,

Abbreviations: CTAB, Cetyltrimethyl Ammonium Bromide; RNA-Seq, RNA Sequencing; NCBI, National Center for Biotechnology Information; NR, NCBI's Non-redundant database; NT, NCBI's nucleotide database; KEGG, Kyoto Encyclopedia of Genes and Genomes; COGs, Clusters of Orthologous Groups of proteins; GO, Gene Ontology; BLASTx, Basic Local Alignment Search Tool for searching protein databases using a translated nucleotide query; DEGs, differentially expressed genes; DEPs, differentially expressed proteins; iTRAQ, isobaric tags for relative and absolute quantification; LC-MS/MS, Liquid chromatography-mass spectrometry; RBH, the reciprocal-best-BLAST-hits analysis; CV, coefficient of variation; S6PDH, NADP-dependent D-sorbitol-6-phosphate dehydrogenase; ANS, anthocyanin synthase; PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase.

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RESEARCH ARTICLE

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High-density genetic map construction and quantitative trait loci analysis of the stony hard phenotype in peach based on restriction-site associated DNA sequencing

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Abstract

Background: Peach (*Prunus persica*) is an important fruit crop that generally softens rapidly after harvest resulting in a short shelf-life. By contrast, stony hard (SH) peach fruit does not soften and hardly produces ethylene. To explore the candidate genes responsible for the SH phenotype, a high-density genetic map was constructed by restriction-site associated DNA sequencing technology.

Results: In the present study, the linkage map consisted of 1310 single nucleotide polymorphism markers, spanning 454.2 cM, with an average marker distance of 0.347 cM. The single nucleotide polymorphisms were able to anchor eight linkage groups to their corresponding chromosomes. Based on this high-density integrated peach linkage map and two years of fruit phenotyping, two potential quantitative trait loci for the SH trait were identified and positioned on the genetic map. Additionally, *Prupe6G150900.1*, a key gene in abscisic acid (ABA) biosynthesis, displayed a differential expression profile identical to the ABA accumulation pattern: mRNA transcripts were maintained at a high level during storage of SH peaches but occurred at low levels in melting fruit.

Conclusion: Thus *Prupe6G150900.1* might play a crucial role in the SH phenotype of peach in which ABA negatively regulates ethylene production. Also, this high-density linkage map of peach will contribute to the mapping of important fruit traits and quantitative trait loci identification.

Keywords: Peach, Stony hard, Restriction-site associated DNA sequencing, Genetic map, Quantitative trait loci, Gene expression

Background

Peach [*Prunus persica* (L.) Batsch] is a typical climacteric fruit, and it generally softens rapidly after harvest, resulting in a short shelf-life that unfavorably affects its market value [1, 2]. Therefore, peach fruit texture is an important quality in the breeding of fresh market varieties.

To date, peach cultivars have been classified into three flesh texture types, melting (M), no-melting (NM) and stony hard (SH) based on the characteristics of fruit firmness and texture changes during peach ripening and

softening [3–5]. Generally, M fruit are characterized by their prominent softening after harvest, NM fruits are characterized by slow softening at the later stages of ripening and never melt, while the SH type does not produce ethylene and barely softens after harvest (both on- and off-tree) [3, 4, 6]. A genetic analysis indicated that the SH genotype is controlled by a single recessive gene [5] and is epistatic to the locus [4].

In general, the softening of dimorphic fruit, is related to endo-polygalacturonase enzyme activity, and is induced by ethylene [7–9]. A low level of ethylene production may contribute to the suppression of fruit softening in SH peach cultivars, and this depends on the suppressed transcription of *PpACS1* [10]. The suppression of *PpACS1* is caused by low indole-3-acetic acid concentrations in SH

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Article

Identifying Genome-Wide Sequence Variations and Candidate Genes Implicated in Self-Incompatibility by Resequencing *Fragaria viridis*

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Abstract: It is clear that the incompatibility system in *Fragaria* is gametophytic, however, the genetic mechanism behind this remains elusive. Eleven second-generation lines of *Fragaria viridis* with different compatibility were obtained by manual self-pollination, which can be displayed directly by the level of fruit-set rate. We sequenced two second-generation selfing lines with large differences in fruit-set rate: Ls-S₂-53 as a self-incompatible sequencing sample, and Ls-S₂-76 as a strong self-compatible sequencing sample. *Fragaria vesca* was used as a completely self-compatible reference sample, and the genome-wide variations were identified and subsequently annotated. The distribution of polymorphisms is similar on each chromosome between the two sequencing samples, however, the distribution regions and the number of homozygous variations are inconsistent. Expression pattern analysis showed that six candidate genes were significantly associated with self-incompatibility. Using *F. vesca* as a reference, we focused our attention on the gene *FIP2-like* (FH protein interacting protein), associated with actin cytoskeleton formation, as the resulting proteins in Ls-S₂-53 and Ls-S₂-76 have each lost a number of different amino acids. Suppression of *FIP2-like* to some extent inhibits germination of pollen grains and growth of pollen tubes by reducing F-actin of the pollen tube tips. Our results suggest that the differential distribution of homozygous variations affects *F. viridis* fruit-set rate and that the fully encoded *FIP2-like* can function normally to promote F-actin formation, while the new *FIP2-like* proteins with shortened amino acid sequences have influenced the (in)compatibility of two selfing lines of *F. viridis*.

Keywords: *Fragaria viridis*; genome; variations; resequencing; self-incompatibility; *FIP2-like*

1. Introduction

Fragaria viridis Weston, known as the green strawberry, belongs to a diploid wild resource of the genus *Fragaria* of Rosaceae and is a potential breeding material for the character optimization of cultivated strawberry. It has a variety of excellent properties, such as firm flesh, remontant flowering habit, good flower characteristics, and an acidic apple-like aroma [1]. Although self-compatibility has been reported in *Fragaria vesca* [2], *F. viridis* has gametophytic self-incompatibility [1,3]. In contrast to the genera *Malus*, *Pyrus*, and *Prunus* [4], *F. viridis* has two controlling sites; thus, the self-incompatibility mechanisms that control the pollen–pistil interaction are difficult to understand [4,5]. Based on manually self-pollinated first generation Ls-S₁-2 of *F. viridis* [6], a group of *F. viridis* second-generation selfing lines with large differences in fruit-set rate was obtained by manual self-pollination. The lowest fruit-set rate was only 2%, while the highest was up to 80%, however, this is still different from the

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Genome-wide discovery and characterization of flower development related long non-coding RNAs in *Prunus mume*

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Abstract

Background: Long non-coding RNAs (lncRNAs) are transcripts more than 200 bp in length do not encode proteins. Up to the present, it has been reported that lncRNAs play an essential role in developmental processes through their regulatory functions. However, their characteristics, expression inheritance patterns, and functions in *Prunus mume* are quite unidentified.

Results: In this present study, we exposed the specific characters of pistil development process between single pistil cv 'Qingjia No.2' (QJN2) and multiple pistils cv 'Da Yu' (DY). We found that early October is the key stage for pistil differentiation. The similarity epidermis was observed between two types of pistil. We also further investigated a complete pistil development lncRNA profiles through RNA-seq in *Prunus mume*. 2572 unique lncRNAs and 24,648 genes mapped to *Prunus mume* genome, furthermore, 591 novel lncRNAs were predicted. Both unique lncRNAs and novel lncRNAs are shorter in length than the mRNAs, and the overall expression level of lncRNAs was lower than mRNAs in *Prunus mume*. 186 known lncRNAs, 1638 genes and 89 novel lncRNAs were identified as significant differential expressed in QJN2 compared with DY. We predicted 421 target genes of differentially expressed known lncRNAs (DEKs) and 254 target genes of differentially expressed novel lncRNAs (DENs). 153 miRNAs were predicted interacted with 100 DEKs while 112 miRNAs were predicted interacted with 55 DENs. Further analysis of the DEKs showed that the lncRNA of XR_514690.2 down-regulated its target ppe-miR172d, and up-regulated AP2, respectively. Meanwhile, the other lncRNA of TCONS_00032517 induced cytokinin negative regulator gene A-ARR expression via repressing its target miRNA ppe-miR160a/b in DY. At the same time we found that the AP2 expression was significantly up-regulated by zeatin (ZT) treatment in flower buds. Our experiments suggest that the two lncRNAs of XR_514690.2 and TCONS_00032517 might contribute the formation of multiple pistils in *Prunus mume*.

Conclusion: This study shows the first characterization of lncRNAs involved in pistil development and provides new indications to elucidate how lncRNAs and their targets play role in pistil differentiation and flower development in *Prunus mume*.

Keywords: lncRNAs, miR172d, Pistil differentiation, *Prunus mume*, RNA-seq, Hormones

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E7-P31

EFFECT OF SALICYLIC ACID ON THE SHELF LIFE OF TOMATO (*LYCOPERSICON ESCULENTUM* L.) CULTIVARS ROMA AND CAMPARI

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Tomato (*Lycopersicon esculentum* Mill) is significant vegetable crop of Pakistan and around the globe. The production of tomato 0.14 million tonnes with export of 11 thousand tonnes of worth 0.47 million rupees. Tomato is a sign of perishable crops of horticulture. Salicylic acid is a prime substance which can be used for enhancing the shelf life of tomato. Cultivars of Roma and Campari were evaluated with effect of five doses of salicylic acid were used from control to 0.1, 0.3, 0.5, 0.7 and 1.0 mM. After the application the produce were place on 4°C for 15 days. It was revealed that 0.5 mM Salicylic acid was stands best in controlling the post shrivelling. While it is also revealed that it good tomato colour is also developed. However both varieties perform equally well on this dose of Salicylic acid. Physiochemical analysis revealed that fruit contain a higher amount of ascorbic acid, lycopene and carotenoids as compared to the control treatment. It is concluded that salicylic acid has a potential to increase the storage life of tomato.



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E29-P43

**EFFECT OF HYDRO AND HALOPRIMING ON THE GERMINATION OF TOMATO
(*LYCOPERSICON ESCULENTUM*) CULTIVARS SUNDAR AND SHAMS**

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Tomato (*Lycopersicon esculentum*) is major crop of the world use for both fresh market and processing purpose. It is significant vegetable crop of Pakistan and around the globe. Local tomato cultivars face the poor germination problem due to improper handling. Seed priming is the effective technique to challenge and enhance the germination process in different environmental conditions. The performance of primed and non-primed seeds during emergence and germination was evaluated in controlled environment RCBD. Two tomato cultivars were selected Sundar and Shams to check the effect of hydro and halo priming. At 25°C±2 priming agents (Distilled water, NaCl, salicylic acid and KNO₃) was used for (0, 8hr, 16hr, and 24hrs). Primed seeds were kept in petri dishes then best emergence time of seeds, germination index, seeding fresh weight, root length, shoot length was recorded. KNO₃ mM at 16 hrs in Sundar cultivar revealed better results than the controlled treatment. Salicylic acid reduced the 50% time of emergence and emergence vigour in Shams cultivar at 24hrs.



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E22-P37

**WATER USE EFFICIENCY FOR VEGETABLE CROP PRODUCTION IN PAKISTAN
UNDER DIFFERENT IRRIGATION SYSTEMS**

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Pakistan is very rich in agriculture and its irrigation system totally depend on the availability of surface water. Improving water quality and productivity can make a big change in global food production and to relief poverty. The present study was done to survey the total water productivity in Punjab using at farm level. Field studies were carried out during 2014–2015 on different field plots to examine total yield, inputs, irrigation, costs and economic return of bitter melon, cucumber and okra grown under drip irrigation vs sugar cane grown under border irrigation. The purpose of this research was to check the appropriate combinations of vegetable crops grown under drip irrigation. Results described that cucumber and okra performed well than bitter melon under drip irrigation system as compared to sugar cane under border irrigation system. Average crop water productivity was expected at 9.7 and 8.2 kg m⁻³ for the cucumber and okra crops, respectively. The cucumber crop gave maximum return of Rs 3, 20,000/acre. It is also noticed that drip irrigation performed well in economic as well as in crop water productivity per cubic meter of water applied. Final results presented a good decision for farmers to grow vegetables at farm level by adopting drip irrigation system.

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found that NAA at 0.5 mg/L (RT4) gave maximum (5.70) leaves per shoot while mean roots per shoot (4.67) was recorded at MS with 1mg/L NAA and 1mg/L IAA (RT3). Maximum mean value (4.67) for roots per shoot was recorded in MS medium supplemented with 1mg/L NAA and 1mg/L IAA (RT3). Maximum mean value (5.58cm) for longest root length was recorded in MS medium 2 supplemented with 1mg/L NAA and 0.5mg/L IAA (RT2) respectively. The genotype Wiallum8818 hybrid gained maximum value for mean leaves per shoot (4.47) while variety Pisang showed maximum value for roots per shoot (4.11) and longest root length (5.48cm).

***In vitro* clonal propagation technology in guava: An overview**

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Guava (*Psidium guajava* L.) cultivation and production is expanding despite biotic stress and growers are diverting towards high density plantations to meadow orcharding systems which demands establishment of better plant multiplication systems producing quality clonal plant material. Sixty elite guava strains in 'Round' (Gola) and 'Pyriform' (Surahi) cultivars were collected across Punjab province and were screened for morpho-physiological traits and great diversity in fruit shape, size, quality and nomenclature was observed in both cultivars. Selected plant material has been established as germplasm unit (GPU) in the Institute Experimental Gardens for further evaluations. Application of 5%-10% sodium hypochlorite controlling deep seated contamination in the nodal cuttings. Higher levels (200-300 mgL⁻¹) of antioxidant poly vinyl pyrrol idone (PVP) effectively reduced phenolic exudation and enhanced explant survival and bud break percentage. Explant browning was less in young juvenile tissues compared with mature woody plant material. Explant position and Genotype dependent multiple shoot induction was noted at higher levels of BAP (3-4 mgL⁻¹) supplemented in Murashige and Skoog media and mother plants were developed for further multiplications. Rooting was achieved either in the same media or media containing 2-3 mgL⁻¹ IBA. The acclimatized plant material has been transferred to green house for further plant growth and development. The

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Trees
Nanjing Agricultural University

**Enhanced in vitro regeneration of rose (*Rosa indica* L.) cv.
American Beauty**

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Abstract: In vitro regeneration of rose (*Rosa indica* L.) is a quick and efficient approach for multiplication to acquire desired traits and vigorous disease-free plants. Nodal sections used as ex-plant and cultured on MS (Murashige and Skoog) medium supplemented with different combination and concentration of BAP, KI, Zeatin and 3% sucrose. Twenty-one days of the nodal section, maximum shoot induction recorded on MS+2.0 mg/l BAP+0.5 mg/l Zeatin. Multiple shoot induction (6-7 shoot per plant) also recorded when these plantlets were sub-cultured on the same medium. For rooting, healthy shoots were excised and cultured on half strength (½) MS medium supplemented with 1.5 mg/l IBA and 1.0 mg/l IAA. Rooting starts after twenty days of transferring to medium while the frequency of rooting was 4-5 roots per plant. Then regenerated young plantlets were successfully acclimatized to the green-house for further growth and experiments.

Keywords: Rose, regeneration, rooting, acclimatization

NOTE

positive chill units; PCU). A 50% bud break was observed in floral and vegetative bud cuttings at 300 and 600 PCU, respectively. A mean time to bud break was inversely proportional to the chilling treatment. The low-temperature stimulated starch hydrolysis accompanied with sucrose accumulation in all organs. Sucrose and sorbitol content increased substantially peaking at 100, 400, and 100 PCU in floral buds, vegetative buds, and bark, respectively, thereafter decreased when buds approached chilling satisfaction (300 and 600 PCU for the floral and vegetative buds, respectively), and then increased again up to 700 PCU. Hexoses (glucose and fructose) accumulated constantly in the buds from 0 to 700 PCU. In bark, glucose and fructose content increased up to 400 PCU, and then gradually decreased. Total amylolytic and α -amylase activities increased in all organs, especially in the floral and vegetative buds up to 100 PCU and then decreased in the floral and vegetative buds before increasing again after endo-dormancy release. Invertase activity remained high in the buds during chilling satisfaction possibly because of translocation of sucrose to the buds which functioned as a strong sink. The results suggest that a low availability of hexoses may be the cause of limited bud breaks due to lack of chilling. Chilling satisfaction of the buds may increase the content of soluble sugars and acid invertase activity, and decrease the starch content, which may correlate with improved bud breaks.

Chip sequencing: an emerging technique for DNA-protein interaction and histone modification

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Chromatin immunoprecipitation using next-generation sequencing (ChIP-seq) is an extensively used technique for studying genome-wide DNA-protein interactions. Due to the tremendous advances in next-generation sequencing technology (NGS), ChIP-seq offers higher resolution, less noise and wide exposure than its array-based precursor ChIP-chip. ChIP-seq has become

High-throughput phenotyping for agricultural research and crop improvement

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Plant phenotyping systems deliver gathering of data on the physical properties at multiple levels. Phenomics and genomics revolutionized the field of agriculture, through captivating researchers to the estimation of complex traits, including crop yield, growth and adaptation to stresses. Furthermore, high throughput phenotyping (HTP) helped to improve and understanding of the crop phenotypes and physiology through most powerful techniques that are practical rather than analytical and analogous to genomic selection (GS). Integration of GS and HTP has the communal empirical approach that assisted plant breeders to use phenotype and genome profile. The benefits of spectral reflectance indices (SRI), based on the Red-Blue-Green digital images as a low-cost device for the estimation of numerous traits (e.g, seed yield, carbon to nitrogen ratio and leaf N concentration) these are greatly valuable for the plant breeders. Similarly, on the basis of classification approaches, PLS-DA; is an effective tool that can identify elite genotypes directly as a substitute to emphasis assessment of estimated trait-values. Furthermore, custom-developed airborne thermography image software (ChopIt), has efficacy that used for the segmentation of plots and estimation of canopy temperature from every single plot by a non-technical user. To test the response of *Arabidopsis thaliana*, in salinity, Plant ScreenTM Compact System (PSI, Czech Republic) equipped RGB imaging, chlorophyll fluorescence used successfully, as well as with robotically weighing and watering of the plants. Hence, the field based effective high- throughput phenotyping systems have become great research tools to help researchers, breeders and developers who work rigorously on biochemical and molecular levels for cultivars development and further adjustable to unlike challenging environmental conditions.

Integrating proteomic and transcriptomic analysis for modern crop improvement

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Advancement in genetics and genomics has become a progressive technique to enhance our understanding related to different plant aspects such as functional and structural analysis for crop improvement. Omic techniques such as proteomics and transcriptomics are the excellent way to study plants function at genome wide level. Through proteomic analysis, we prolonged the area of protein biochemistry that includes database of protein sequences, predicted protein structures and, protein expression analysis. While transcriptomic analysis use micro or macro arrays-based sequencing methods for expression profile at genomic level. Through these analyses, we can identify the relevant function of our gene of interest involved in crop improvement. Therefore, we need to integrate disciplines such as structural genomics, proteomic, transcriptomic and different metabolomics for breeding assistance. Hence, we can say that high-throughput approaches contribute in genomic research for crop improvement.

Screening of 'pyriform' guava strains for physico-chemical attributes

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Twenty-eight strains of guava (*Psidium guajava* L.) cultivar Pyriform (Surahi) were collected from geographically distant localities in Punjab-Pakistan and evaluated for physico-chemical properties to select better candidates for clonal propagation and breeding programs. The largest fruit were observed in S12 (MSSP; 91.75 mm) followed by S26. The widest fruit was noted in S10 (MSAW; 70.84 mm) followed by S12. Maximum ratio FL/FD was observed in accessions S26 and S12. Maximum flesh thickness was found in S10 (MSAW; 39.18 mm) followed by S26. Maximum fruit weight and flesh weight were recorded in S7 (MSSM; 214.13 g, 182.51 g) followed by S10. In different strains, SCW ranged from 14.04 g to 32.47 g. Maximum seed weight was found in S20 (GSSM; 2.45 g) while minimum weight was recorded in S28 (DSSM; 0.90 g). Maximum number of seeds were found in S20 (GSSM; 267.40) while minimum seeds were noted in S28 (DSSM; 99.60). Maximum TSS was found in S12 (MSSP; 9.62 °Brix) followed by S15. Maximum acidity was found in S12 (MSSP; 0.72%) while S24 (CSAW; 0.23%) showed minimum TA. Maximum ratio TSS/TA was found in S24 (CSAW; 37.05) followed by S1. Maximum ascorbic acid content was found in S14 (SBSSP; 1.92 mgml⁻¹) followed by S23. Maximum total sugars were found in S14 (SBSSP; 6.45%) followed by S21 (CSAW; 6.43%). Principle Component Analysis (PCA) analysis revealed that fruit size, fruit weight and flesh weight largely contributed for variations. Strains DSSM and MSSP were found most divergent and outliers. The dendrogram showed three main groups with eight sub-groups showing variation within strains and among different strains. These studies indicate enormous potential in 'Pyriform' guava strains for selection, clonal propagation and exploitation as parental material for breeding programs.

**Silver nitrate changes the physiology of reproductive system in
parthenocarpic cucumber**

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In Pakistan, almost all the vegetable seed especially hybrid seed of parthenocarpic cucumber import every year. So, keeping in mind that, gynoeious cucumber seed must produce locally, work was started in Vegetable Research Institute Faisalabad, Pakistan in winter 2016. To start breeding in parthenocarpic cucumber, first requirement was to induce male flower. For this purpose, five cucumber gynoecious lines were planted under high tunnel, facilitated with drip irrigation system, insect proof and white plastic sheet. When, the plants were at the first true leave stage, fifteen plants, five for each treatment; 0.01%, 0.02% and 0.03% silver nitrate concentration were selected. Plants were sprayed on their epical meristem every two days interval with 5 times applications. These treatments were conducted in three replications. After 5 applications, the plants treated with 0.01% solution, did not show any change in their physiology and phenotype. The plants treated with 0.02% solution first showed hermaphrodite flowers, there male pollen was non-viable, but from 2nd node produced male flowers primordia in the axil of leaves and their growth was stunted for some days. Furthermore, plants treated with 0.03 % solution stopped their vegetative growth. After a few days, the plants treated with 0.03 % treatment died, while the plants treated with 0.02% solution showed male flowers that were used in the cross-pollination. These plants produced male flowers till 22 days after 5th-time application of 0.02% solution. After 26 days, plants again shifted towards their normal behavior,