Biochemical and molecular characterization of diverse genotypes of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties

Thesis

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By

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August 2018
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CERTIFICATE

This is to certify that the thesis entitled, **"Biochemical and molecular characterization of diverse genotypes of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Horticulture** and minor in **Molecular Biology and Biotechnology** of the college of Post Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar is a record of bonafide research carried out by **Ms. Anjana Kholia, Id. No. 38105** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation and source of literature have been duly acknowledged.

August, 2018
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CERTIFICATE

We, the undersigned members of the Advisory Committee of **Ms. Anjana Kholia, Id. No. 38105**, a candidate for the degree of **Doctor of Philosophy** with major in **Horticulture** and minor in **Molecular Biology and Biotechnology** agree that the thesis entitled **"Biochemical and molecular characterization of diverse genotypes of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties"** may be submitted in partial fulfillment of the requirements for the degree.

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ABBREVIATIONS

%	Per cent
/	per
:	Ratio
°C	Degree celsius
Δ**O.D.**	Change in optical density
µmole	Micro mole
µg	Micro gram
µl	Micro litre
bp	Base pair
CAT	Catalase
CD	Critical difference
CRD	Completely Randomized Design
dNTPs	Deoxynucleoside triphosphates
DPPH	2, 2- diphenyl-1-picrylhydrazyl
Dw	Dry weight
et al.	*et alia* (and others)
FCRD	Factorial Completely Randomized Design
Fig.	Figure
Fw	Fresh weight
g	Gram
GAE	Gallic acid equivalent
i.e.	That is
mg	Milli gram
min.	Minute
mM	Micro mole
mm	Millimetre

ng	Nano gram
NS	Non significant
O.D.	Optical density
PAL	Phenylalanine ammonia lyase
PCR	Polymerase chain reaction
PIC	Polymorphic information content
pM	Pico mole
POD	Peroxidase
PPO	Polyphenol oxidase
S.Em.±	Standard Error of Mean
SOD	Superoxide dismutase
SSR	Simple sequence repeats
TCA	Trans cinnamic acid
U	Unit

Introduction

Chapter 1 INTRODUCTION

Bael (*Aegle marmelos* Correa.) is an incredible minor fruit crop indigenous to India. It has been known to India from prehistoric times. It has great mythological and religious significance in Indian history and culture. It is considered as a sacred tree, its trifoliate leaf offered to lord Shiva. The leaves are ternate and hence one of the vernacular names is 'Tripatra'. Bael tree has wide adaptability to adverse climatic and soil conditions, can tolerate high soil salinity. Therefore, consider as pertinent fruit crop for arid and semi-arid region. In order to achieve increased production from waste lands, arid and semi arid regions, Bael is very suitable with minimum inputs. Beside, the horticultural importance of Bael, owing to its environment friendly nature, is being placed among plant species group called "climate purifiers" as it emits a greater percentage of oxygen during day time as compared to other plants **(Agarwal, 1997)**. This tree is mentioned in the pre-historic writings dating back to 800 B.C. The Buddhist pilgrim, Hiuen Tsiang, when came to India (1629 A.D.), noticed the presence of this tree in India. The Bael trees are found in many South East Asian countries including Pakistan, Sri Lanka, Nepal, Myanmar, Bangladesh, Vietnam, Cambodia Thailand, Malaysia, Java, Philippines and Fiji. It been also been introduced to Florida (U.S.A.). It grows well in the dry forests on hilly and plain areas. The slow growing and spiny trees of Bael are usually found in waste lands and generally used as a wind break near the field boundaries. It is found almost in all the states of India such as in Andhra Pradesh, Bihar, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, Uttarakhand and West Bengal. In India, it is cultivated, traded and consumed locally.

The genus *Aegle* belongs to the order Sapindales, family Rutaceae, subfamily Aurantioideae, tribe Clauseneae and subtribe Balsamocitrinae. The generic name *Aegle* is of greek origin and the species *marmelos* is of Portuguese origin. Meiotic studies revealed the existence of both intraspecific diploid (2n = 18) and tetraploid cytotypes (2n = 36). Tree is deciduous in nature, 6-8 meters in height with trifoliate aromatic leafs. Its bark is thick, branches are spiny in some varieties, and the lower ones are drooping. New emerging foliage is glossy, shiny and pinkish maroon. Flowers are

bisexual, fragrant having sweet aroma and blooms in clusters of 4 to 7 along the young branchlets. Each flower has 4 curved fleshy petals which are green outside and yellowish inside, and 50 or more greenish-yellow stamens. Fruit may have a thin, hard, woody or soft rind. It is dotted with minute oil glands which are aromatic. Inside the fruit, there is a hard central core and 8 to 20 faintly defined triangular segments, with thin, dark orange walls. These segments are filled with aromatic, pale orange, pasty, sweet, resinous, more or less astringent pulp. 100 to 150 seeds are embedded in the fruit pulp. Seeds are flattened-oblong, about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying. The shapes of fruits can vary with varieties and can have round, pyriform, oval, or oblong in shapes having 5-20 cm diameter. Its fruits are botanical hard shelled berries. Its fruits are green at early immature stage and turn yellow when mature.

Bael fruits are highly nutritious and very good source of vitamins, minerals, fiber and pectin. It contains 61g moisture, 1.6g protein, 0.2g fat, 1.9g mineral, 80mg calcium, 52mg phosphorus, 0.5mg Iron, 0.20mg copper, 31.8g carbohydrate, 2.9g fiber, 55mg carotene, 0.13mg thiamine, 1.19mg riboflavin, 1.1mg niacin, 8mg vitamin C per 100g of edible portion. Its food value is 88 calories/100gm. Along with the nutritional significance; it is a wonderful ethanobotanical herb. Bael has been used in the Indian traditional medicines from time immemorial. It is associated with various important medicinal properties. All parts of this tree, *viz.,* root, leaf, trunk, fruit and seed, are used for curing one human ailment or another. The chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites. Bael fruit consists of considerable amount of flavoring compounds and many bioactive compounds. Its root, fruit, bark and leaf contain alkaloids, coumarins and furocoumarins such as aegelenine, aegeline skimmianine, marmelosin, marmesin and scopoletin. The unripe fruit is an astringent, a digestive and stomachic, and is used to cure diarrhoea and dysentery. The unripe fruit has been reported to show antiviral activity and proved to be potent hypoglycemic agent **(Shinde *et al.,* 2014)**. The roots and bark are used in the treatment of fever and to control pain in the abdomen. The leaves possess anti-inflammatory and analgesic properties. The plant has been widely used for its having antibacterial, antifungal, antioxidant, antidiarrhoeti, pesticidal, antipyretic, antidote and anti-inflammatory properties. Various parts of the plants are

also used for treating anaemia, wound healing, high blood pressure, asthma, jaundice, and troubles during pregnancy, typhoid, hepatitis, tuberculosis, dyspepsia, diabetes. The medicinal and nutritive properties together with its hardy nature make Bael an ideal fruits for future.

A wide range of diversity in Bael has been noticed in dry subtropical belt of northern India. The Bael cultivars have been identified and found useful for commercial cultivation are NB-5, NB-7, NB-9 Pant Aparna, Pant Sujata, Pant Shivani and Pant Urvashi, CISH-B1, CISH-B2, Goma Yashi, Thar Divya and Thar Neelkanth. The heterozygous nature of Bael leads the great variability, so proper identification and characterization are highly required for the conservation and sustainable use of plant genetic resources. The genetic variation is necessary for long term survival of any species and it is a very critical feature in conservation. To expedite the any crop improvement programmes, it is essential to identify and trap the natural variability and the variability should be conserved *ex situ* and *in situ* to utilize in the further hybridization programmes. Proper study is necessary to build up the understanding of this crop so that sustainable efforts can be put forward to enhance production, accelerate research and develop capabilities to make bael as a profitable enterprise amongst fruit growers of the in India. A number of methods are available for analysis of genetic diversity in genotypes, germplasm accessions, breeding lines and populations. The morphological traits have been used as primary tools to identify and characterize germplasm resources in past. However, due to the effects of environmental factors on these attributes, their use can be ambiguous and differentiation of cultivars through morphological features is inefficient and inaccurate. In contrast to morphological markers, the biochemical and molecular markers are emerged as powerful tools for proper characterization of genetic diversity.

The antioxidants are the substances that protect cells from the damages caused by the unstable molecules known as free radicals. In our body generation of free radicals take place when normal biochemical reactions, increased exposure to the environment, and higher levels of dietary xenobiotics. The reported chemical evidence suggests that dietary antioxidants help in disease prevention. The primary enzymatic antioxidants are superoxidase dismutase, catalases and peroxidase. Beside, these primary enzymatic antioxidants other substances *i.e.,* vitamins, phenolic compounds,

flavonoids, carotenoids, coumarins and pectin posses the antioxidant acitivity. Bael fruit pulp contains all these potential antioxidants. So there is need to characterize these antioxidants present in diverse genotpe of Bael for their further nutraceutical use. The one of the aim of this work was to perform the biochemical characterization of Bael genotypes related with antioxidant activity evaluating the genetic diversity and selecting those genotypes with desirable biochemical qualities for developing a nutraceuticaly rich product and as parents in future breeding programs.

In addition to morphological and biochemical markers, DNA markers can also be used for differentiating various genotypes. They provides genetic basis of diversity, which is more accurate and reliable as compared to morphological and biochemical markers. Many of the complications of a phenotype-based assay can be overcome through direct identification of genotype with DNA-based genetic markers. Therefore, markers independent from the environment are necessary for reliable identification and discrimination of genotypes and cultivars. DNA based molecular markers are independent from environmental interaction, developmental stage, unlimited in number and show high level of polymorphism. Morphological traits are traditional phenotypic markers for the identification of plants. They may change with the cultivation and growing environment and make the identification confusing. Therefore, molecular markers are considered as powerful tools in the assessment of genetic diversity within and between plant populations. The characterization of genetic variations within natural population and among breeding lines is very crucial for effective conservation and exploitation of genetic resources for crop improvement programme. Molecular markers have proven useful for assessment of genetic variation in germplasm collections.

In case of Bael very few molecular markers such as Random Amplified Polymorphic DNA (RAPD) have been developed to study the genetic diversity. Few attempts related to RAPD by **Nayak *et al.,* 2013**; SSR by **Shrama and Sharma, 2015**; ISSR by **Mujeeb *et al.,* 2017** and RAPD by **Misra and Singh, 2008** markers have been made to study the genetic diversity of Bael genotypes. Simple sequence repeats (SSRs) is an efficient genetic markers for comparative genome mapping can be helpful in the classification of genotypes, germplasm resource utilization and breeding programmes. Among the different types of markers, Simple Sequence Repeats (SSRs) are useful for assessment of genetic diversity. Simple sequences repeat (SSRs) molecular maker, as a

new type of DNA molecular marker, the second generation based on the polymerase chain reaction (PCR), is valuable and of great potential as genetic markers. Simple Sequence Repeats (SSRs) are becoming the markers of choice in genetic studies because they are transferable, multiallelic, co-dominant markers, easily reproducible, randomly and widely distributed along the genome. In bael, very scanty information is available on use of both biochemical and molecular markers together for diversity analysis.

The dietary supplements are now made using different fruits, vegetables and also herbs in order to provide natural curative as well as preventive methods for combating diseases and poor health. Because of the importance of Bael fruits as valuable fruit and medicinal resource, many studies have been undertaken to identify, characterize and differentiate various locally grown cultivars of Bael in terms of their proximate fruit composition, antioxidant activity and phytochemical properties of the fruits. Bael is popularly known and appreciated by consumers due to its organoleptic characteristics. However, there is high interest to develop such value added product from Bael that provides benefits beyond basic nutrition for better health. Therefore, considering the above facts and constraints, the present study was undertaken to evaluate Bael genetic diversity under tarai condition with the following objectives:

1. To characterize the variability based on biochemical attributes.
2. To characterize the variation among different genotypes and cultivars of Bael based on SSR polymorphic analysis.
3. To prepare the appetizer from different nutraceuticaly rich genotypes of Bael for quality and nutrition

Review of Literature

Chapter 2 REVIEW OF LITEATURE

In view of the large variability available in Bael, the present investigation has been undertaken to identify some good genotypes based on biochemical and molecular characterization. Along with the biochemical and molecular characterization a study on development of appetizer with nutraceutical potential was under taken. With the emergence of concepts of functional foods (health promoters), there is high interest to study and to quantify the nutraceutical important biochemical components of fruits. Keeping this interest in view, in this chapter a brief review of previous work related to Bael and other fruit crops have been presented under appropriate heading and subheading.

2.1. Biochemical characterization

2.2. Molecular characterization

2.3. Development of nutraceutically rich appetizer from Bael

2.1. Biochemical characterization

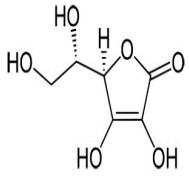
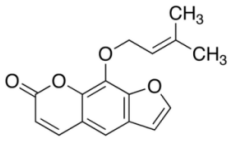
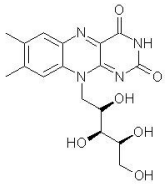
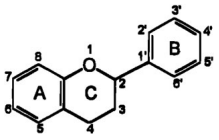
The antioxidants are molecules that inhibit the oxidation of other molecules by interacting with free radicals. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Free radicals reactive oxygen species and reactive nitrogen species are generated in body by various endogenous systems, exposure to different physiochemical conditions or pathological states. Free radicals adversely alter lipids, proteins, and DNA and trigger a number of human diseases. A balance between free radicals and antioxidants are necessary for proper physiological function. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Hence application of external source of antioxidants can assist in copying this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole had been reported to be dangerous for human health **(Grice, 1988 and Wichi, 1986)**. Thus, there is need of identifying more effective, non toxic natural antioxidants. The antioxidants are classified into two groups on the basis of their enzymatic nature: **(a)** Non enzymatic antioxidants: e.g.- Vitamin C, carotenoids and flavonoids **(b)** Enzymatic antioxidants: e.g.- SOD, CAT, POD.

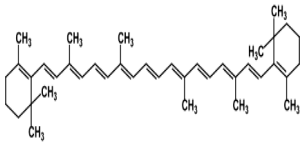
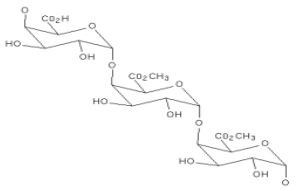
Therefore, in biochemical characterization the main focus was given to evaluate different non enzymatic and enzymatic antioxidant present in Bael fruits. The biochemical characterization reviewed in this chapter is categories into following subheadings.

2.1.1. Non enzymatic antioxidants:

The different non enzymatic antioxidants with their antioxidant acitivity was presented in Table 2.1, and review on non enzymatic antioxidants present in Bael and other fruit presented in following subheadings.

Table 2.1 : Non enzymatic Antioxidants and their antioxidant activity

S. No.	Antioxidant name	Basic structure	Antioxidant activity	Refrences
1.	Ascorbic acid		(i) Scavenge variety species of ROS. (ii) Give off Semi dehydroascorbic acid (iii) Clear $^1O_2$. (iv) Reduce sulfur radicals.	**Amitava and Kimberly (2014)**
2.	Marmelosin		(i) Modulate both oxidative. stress and inflammation effectively.	**Pynam and Shylaja (2018)**
1.	Vitamin B_2		(i) Protect the body against oxidative stress, especially lipid peroxidation and reperfusion oxidative injury.	**Ashoori and Saedisomeolia (2014)**
5.	Phenolic compounds and falvonoids		(i) Inhibit the body's oxidant enzymes. (ii) Improve the body's antioxidant enzyme activity. (iii) Scavenge ROS directly. (iv) Anti-lipid oxidation *in vitro.* (v) Decrease quality of peroxide formation *in vivo.*	**Nakao *et al.*, (2011)**

4.	Carotenoid		(i) Protect the skin against photooxidative damage. (ii) Singlet oxygen quenching. (iii) Peroxyl radical scavenging.	**Stahl and Sies, (2003)**
5.	Pectin		(i) Enhance endogenous antioxidant enzymes. (ii) Disposal of free radicals.	**Koriem *et al.,* (2014)**

2.1.1.1. Ascorbic acid

Ascorbic acid is also known as 'Vitamin-C'. It is a water soluble vitamin present in fruits and other food item and used as a dietary supplement. The deficiency of ascorbic acid lead the disease scurvy, which is prevented and treated with vitamin C containing foods or dietary supplements. Fruits are the rich source of ascorbic acid. The variation in ascorbic acid content was recorded in various genotypes of Bael which ranged from 5.37 – 18.4 mg per 100 g pulp **(Teaotia *et al.*, 1963)**. Much variation in ascorbic acid content of Bael amongst the seven genotypes was reported by (**Jauhari *et al.*, 1969)**. An experiment was laid out to evaluate these commercially important cultivars. Maximum ascorbic acid recorded in 'Narendra Bael-5' **(Srivastava and Singh, 2004)**. The ascorbic content varies significantly among 7 Bael cultivars and recorded maximum in 'NB-5' and minimum in 'Pant Urvashi' **(Saroj *et al.,* 2008)**. Ascorbic acid content were measured in fruit from six genotypes of *Actinidia chinensis*, maximum ascorbic acid is found in genotype CK66_02, whereas minimum is found in genotypes CK64_12 **(Rassam and Laing, 2005).** An evaluation study of fruit physicochemical properties of peach cultivars illustrated that, highest vitamin C obtained from 'Khounihaste- joda' and 'Kosari', whereas lowest vitamin C obtained from Anjiri-ye-maleki' and 'Anjiri-ye-khouni' **(Hajilou and Fakhimrezaei, 2011)**. In a variability studies in aonla wild genotypes for fruit character from the north-eastern region of India, it was revealed that vitamin C content was higher in genotype 'T12'subsequently by 'T19', 'T16' , 'T2' and for the same least value was calculated in genotype 'T20' , 'T

7' and 'T15' **(Singh and Singh, 2016)**.There was significant difference in ascorbic acid content among *Zizyphus* genotypes. The ascorbic acid content depicting five-fold variations among these genotypes. Maximum value was obtained from Chuhara, while the minimum was from Umran (**Koley *et al.,* 2016)**.The physico-chemical profiling of different mandarin cultivars showed that, maximum ascorbic acid was found in Mandarin thorny while the lowest ascorbic acid content was recorded in 'Dancy' **(Varane *et al.,* 2016)**. Among all the cultivars studied 'Langra' possessed the higher amount of ascorbic acid content followed by 'Pusa Surya' and 'Pusa Arunima', however, lower in 'Vanraj' and 'Sabri' (**Bora *et al.,* 2017)**. Among the different strawberry genotype, the highest amount of vitamine C was noted from 'Cristina', while 'Sonata' showed the lowest amount **(Zhong *et al.,* 2017)**. In a study evaluation of Beal genotypes for biochemical characters, highest in ascorbic acid found in 'Acc.1' and lowest in 'Acc.15' **(Pavani *et al.,* 2018)**.

2.1.1.2. Marmelosin

Many naturally occurring phytochemicals with high therapeutic potential has founds in fruit, out of which one important naturally occuring coumarins 'marmelosin' found in different part of Bael. Marmelosin also called as imperatorin is a major chemical constituent of the Bael fruit and has been proved to have an anticancer, antibacterial and anti-inflammatory activity. Beside this, it is antidiabetic agent **(Prajapat *et al.,* 2012)**, it possess anthelmintic activity **(Venugopal *et al.,* 2013)**, hepatoprotective and antidiarrhoel activities **(Shailajan *et al.,* 2012)**.

2.1.1.3. Total Phenol content

Phenol constitutes probably the largest group of plant secondary metabolites, which varying in size from a simple structure with an aromatic ring to complex ones. Phenolic compounds are widely distributed in plant tissues, particularly contributing colour, flavour, and astringency to fruits. Phenolic compounds were extracted from different fruits with mounting evidence suggests that these compounds confer a wide range of health benefits. The phenol content in fruits may vary with genotypes. The fourteen red-fleshed plum and eight peach genotypes were characterized for their total phenolic and significant was differences observed (**Cevallos-Casals *et al.,* 2006).** A previous studies in apple confirmed the difference between apple varieties for total

phenolics **(Khanizadeh *et al.,* 2008).** The eleven cultivars of banana from southern India were analyzed for total phenolic content and it were found to be highest in 'Rasbalei' banana **(Deshmukh *et al.,* 2009)**. The phenolic content of strawberry genotypes compared and it was found, 'Maletto' strawberries had the highest total phenolic content whereas values for 'Tudla' strawberries were lower **(Panico *et al.,* 2009)**. The highest values of total polyphenols were determined in apricot genotypes and 'LE-2527', and the lowest levels in genotypes 'LE-985' and 'LE-994' by **Sochor *et al.,* (2010).** In an experiment entitled "Fruit quality and phytochemical attributes of some apricot cultivars as affected by genotypes and seasons" it was found that, the new hybrid cultivar 'Çagataybey' contained the highest total phenolic among the other cultivars **(Caliskan *et al.,* 2012)**.A significant difference was found for total phenolics content among the tested Iranian apple genotypes. The flesh tissue of 'Hajie karaj' and 'Heidarzade' have the highest phenolic content , while 'IRI1', 'IRI4' and 'IRI6' showed the lowest ones **(Javdani *et al.,* 2013)**. **Kharub *et al.,* (2014)** revealed that the different varieties of Bael exhibited considerable physico-chemical attributes of Bael fruits. The total phenols content of different Bael varieties is ranges from 2.34-2.75%. The variation was observed in pineapple genotypes for total phenolic content. The pineapple genotype 'MD-2' indicated the highest total phenolic content, whereas predominant cultivars 'Comte de Paris' and 'Smooth Cayenne' indicated lowest total phenolic content under China conditions **(Lu *et al.,* 2014)**. Differences in phenolic content among different genotypes were found in oleaster genotype. Maximum phenolic content obtained in genotype 'IEa-4', whereas genotype 'IEa-1' contain minimum (**Faramarz *et al.,* 2015**). The total phenolics content were found to be vary significantly among Indian jujube cultivars. Maximum total phenolics content was obtanined from cultivar 'ZG-3', whereas minimum total phenolics content was obtanined from cultivar 'Chuhara' under Bikaner conditions (**Koley *et al.,* 2016**). Total phenolic content of fruits from Brazilian genotypes of feijoa were determined. The greatest values for Total phenolic content was obtained for 'Mattos' and 'Nonante', in the skin, and for 'Nonante' and accession 2316, in the flesh **(Amarante *et al.,* 2017).** The concentration of total phenols of five peach genotype 'Cascata 962', 'Cascata 1070', 'Conserva 681', 'Conserva 871' and 'Âmbar were highest, whereas the peach genotype 'Libra', 'Tropic Beauty', 'Bonão', 'Cascata 1063', 'Tropic Snow', 'Conserva

1396', 'Cascata 1303' 'Rubimel', 'Conserva 985', 'Conserva 1153', 'Cascata 967', 'Conserva 844', 'Conserva 1129', 'Conserva 1434', 'Conserva 1186', 'Cascata 587') had the lowest concentrations of total phenols (**Fabiane *et al.,* 2017**). The mandarin genotype 'Manju', 'Karamandarin', and 'Parson special mandarin' were generally the genotypes having higher phenolic contents in tissue of fruits. On the contrary, the fruit tissues of 'Shagan' had a lower phenolic content (**Hua *et al.,* 2018**).

2.1.1.4. Pectin content

Pectin are the polysaccharides and major component of the middle lamella, where it helps to bind cells together, but it is also found in primary cell walls. The word 'pectin' derived from the Greek word '*pektos'* which means firm and hard, reflecting the pectin's ability to form gels. Pectin is generally regarded as one of the safest and most acceptable of food additives. Fruit contains large amounts of pectin. The highest value of pectin was found in the 'Winesap' variety and the lowest in ' Lowry' apples **(Lopez *et al.,* 1958)**. Among the different banana, the highest pectin content was measured in 'Rasballe' followed by 'Jawari', 'Mysore' and 'Shrimanti', whereas the lowest was observed in 'Budhbale' and 'Deshi' **(Deshmukh *et al.,* 2009)**. The pectin extracted from different varieties of apple and it was found that the yield of pectin content extracted was predominant in 'Maharaj-ji' followed by 'Delicious' and 'American' **(Mathur *et al.,* 2011)**. The pectin content differed significantly among the guava variety es. 'L-49'was on top of the list for pectin content closely followed by 'Lalit'. These variations may be due to the genetic makeup of the cultivars **(Singh *et al.,* 2016)[a]**.

2.1.1.5. Riboflavin

Riboflavin is known as 'Vitamin B_2'. It is found in food and used as a dietary supplement. It is water soluble vitamin. In a nutritional analysis of two local varieties of papaya noted that, 'Local-2' varieties contains highest riboflavin at ripen stage, whereas the mature stage of 'Local-1' contains lowest riboflavin **(Bari *et al.,* 2006)**. The riboflavin content study of banana cultivars from 'Makira', Solomon Islands revealed that, all Fe'i cultivars and one Non-Fe'i cultivar contained riboflavin. The assessment of these Fe'i cultivars confirmed that 'Karat' has substantially greater riboflavin content **(Englberger *et al.,* 2010)**. Seven major almond varieties (Butte,

Carmel, Fritz, Mission, Monterey, Nonpareil and Sonora) grown in California studied for riboflavin. The highest riboflavin found in 'Butte' and 'Sonora' **(Yada *et al.,* 2013)**.

2.1.1.6. Total carotenoids

Carotenoids are the organic pigments produced by the plants. They are derivatives of tetraterpenes and causes the compounds to be deeply colour yellow, orange, or red. It serves two key roles in plants *i.e.,* they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage *(***Armstrong and Hearst 1996)**. Beside this carotenoids exhibit great antioxidant potential. They are found in abundance in fruits and illustrate great variation. An experiment conducted on "Carotenoids in yellow- and red-fleshed papaya" it was found that, the carotenoid profile and organisation of carotenoids in the cell differ in the two varieties **(Udumalagala Gamage *et al.,* 2003)**. The thirty-seven apricot varieties, including four new releases (Rojo Pasion, Murciana, Selene, and Dorada) obtained from different crosses between apricot varieties and three traditional Spanish cultivars (Currot, Mauricio, and Bulida), of apricot were studied for carotenoids content. The maximum total carotenoid obtained from orange flesh apricot variety, whereas, minimum total carotenoid obtained from white flesh variety **(Ruiz *et al.,* 2005)**. The great differences in carotenoid content among banana cultivars were observed. The maximum carotenoid content found in yellow-orange fleshed cultivar 'Fe'i Aibwo/Suria', whereas , minimum in the white-fleshed 'Non Fe'i Saena' **(Englberger *et al.,* 2010)**. Among the different mango varieties examined for carotenoid content, 'Amrapali' contains the highest amount of carotenoid, whereas 'Surmi Fazli' contains the lowest amount of carotenoid under Bangladesh condition **(Haque *et al.,* 2015)**. In another study the maximum carotenoids content was observed in 'Amrapali' followed by 'Mallika', whereas the minimum in 'Mahmood Bahar' under Pantnagar condition **(Bora *et al.,* 2017).**

2.1.1.7. Falvonoids

In a study on flavonoid contents in fruits of different of *Citrus* genotypes, it was found that the more abundant content was registered in 'Red Blush', followed by 'Oroblanco', whereas the more scarce was found in 'B-92' **(Germana *et al.,* 2004)**. The significant difference for total flavonid content found between the tested apple genotypes fruit. The flesh tissue of 'Heidarzade' and 'Soltanie shabestar' fruits had the highest

content of total flavonoid, while 'IRI1' and 'IRI4' had the lowest ones **Javdani *et al.,* (2013)**. The values of total flavonoid content in pineapple genotypes varied significantly, highest total flavonoid content obtained in 'Smooth Cayenne #1', whereas the lowest in 'Comte de Paris' **(Lu *et al.,* 2014)**. A variation in terms of total flavonoid content was observed among oleaster genotypes results showed in flesh, it was found maximum in 'IEa-1' and minimum in 'IEa-7' (**Faramarz *et al.,* 2015**). The content of total flavonoids was also found to vary significantly among *Zizyphus* genotypes, maximum content found in 'Gola' and minimum in 'Chuhara' **(Koley *et al.,* 2016)**. The mandarin 19 genotypes studied, the 'Karamandarin' had higher flavonoid contents of fruit tissues than other genotypes. On the other hand, 'Shagan' and 'Avanaapireno' were the ones with a relatively lower flavonoid content in fruit tissues **(Hua *et al.*, 2018)**.

2.1.2. Enzymatic antioxidants

In an investigation activity of all antioxidant enzymes evaluated in acerola pulp and found that CAT activity was the highest among the evaluated enzymes for the three acerola fruit varieties and it also declined significantly with development. As for the other two evaluated enzymes, peroxidise activity was higher in 'Flor Branca' fruit **(De Souza *et al.,* 2014)**. The apple peel tissue exhibited significantly higher antioxidant enzymes (SOD and APX) activities, with the exception of CAT (**Li *et al.,* 2014**). The 'Pink Lady' apple cultivar fruit exhibited appreciable levels of SOD and CAT activities harvested during extended period of fruit harvest maturity and subsequent fruit ripening **(Abbasi *et al.,* 2010)**. In an investigation evaluate the enzymatic activity of peroxidase in avocado pulps, from the Northwest area of Paraná-Brazil, in order to compare the varieties on their enzymatic activity for both, minimum and industrial processing. Enzymatic extracts were prepared from avocado pulp of Choquete, Fortuna and Quintal varieties, in green and ripe maturation stage. Soluble peroxidase showed activity in the green stage, whereas, ionically bound peroxidase activity increased with the change from green to ripe maturation stage in Choquete variety **(Vanini *et al.,* 2010)**. The activity of catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), were determined in ten wild edible fruits. The CAT and SOD activity was increased in mature fruits than ripened fruits, whereas POX activity found to be more in ripened fruits as compare to mature fruits (**Valvi *et al.,* 2011**) Behavior of enzymatic antioxidant system during development and ripening of cashew apples was studied. The

clones analyzed included: CCP 76, CCP 09, BRS 189 and BRS 265 in seven development and ripening stages. Superoxide dismutase (SOD) activity showed an increase during fruit ripening in CCP 09 clone at end of storage, but it remained lower in CCP 76 than in the other cultivars. CCP 09 clone showed the CAT activity 3-fold less than BRS 265 at end of storage **(Lopes *et al.,* 2015)**.

2.1.3. Other biochemical parameters

2.1.3.1. DPPH radical scavenging (%)

Antioxidant potential of pomegranate cultivars grown in Sri Lanka were studied and found that the 'Nayana' cultivar showing the highest antioxidant activity followed by the Indian cultivar 'Nimali' and 'Daya' **(Bopitiya and Madhujith, 2012)**. Variation in the DPPH values obtained from antioxidant assays of Banana Cultivars from Southern India **(Deshmukh *et al.,* 2009)**. In an experiment entitled "Bioactive compounds and antioxidant activity during maturation of strawberry guava fruit", it was observed that, The lowest antioxidant activity for the completely green stage was seen in the 'P14' genotype. For the remaining stages lower values were observed in the fruit of genotype 'P9'. In contrast, those genotypes that showed the greatest antioxidant activity for the four stages evaluated were 'P4', 'P6' and 'P7' and were indicated as being the most promising **(Dantas *et. al.*, 2013)**. Antioxidant activity determined by the DPPH in pineapple genotypes, maximum value is obtained from 'MD-2', while the genotype 'CPM' showed the minimum value **(Lu *et al.,* 2014)**. The highest DPPH radical scavenging activity was observed in the flesh extract of 'IEa- 1', whereas the lowest radical scavenging activity was noted in flesh extract of 'IEa- 4' in oleaster genotype **(Faramarz *et al.,* 2015)**. The antioxidant capacity variation of the pulp residues among 19 citrus genotypes is studied. It was revealed that the DPPH values varied with 'Shinamanatsu', 'Karamandarin', 'Hayaka', 'Tankantangor', 'Yuanhongxianggan' having the highest , whereas 'Baiju' genotype having the lowest **(Hua *et al.,* 2018)**.

2.1.3.2. TSS(Total soluble solids)

TSS of Bael pulp varied from 28-36 per cent **(Jauhari *et al.* 1969)**. Great variation in total soluble solids (32-34%) of Bael fruits was recorded **(Jauhari and Singh, 1971)**. TSS ranging from 31.0 per cent to 35.5 per cent was noted **(Roy and Singh, 1978)**. In study on variability on physico-chemical characters of Bael fruits of 8

selected genotypes in Uttar Pradesh, significant variations reported in TSS among various genotypes **(Singh *et al.*, 2000)**. Variability reported in physico-chemical quality of Bael in four cultivars viz., 'Narendra Bael-4' 'Narendra Bael-5'.'Narendra Bael-7' and 'Narendra Bael-9'. Of these, maximum total soluble solids was recorded in 'Narendra Bael - 7' **(Srivastava and Singh, 2004).** The twenty one Bael Genotypes from Orissa showed considerable variations with respect to physico-chemical characters assessed. The maximum total soluble solid (40.43^{o}Brix) of fruit was recorded in CHBl-20 **(Kumar *et al.*, 2010)**. Collection and evaluation of Bael genotypes were carried out by **Mitra *et al.*, (2010)**. They observed more variability in total soluble solids ranges from 23–42 °Brix among genotypes. The clones T1, T5, T8, T10, T15 and T16 were selected for cultivation. In another study on Evaluation of Bael varieties under rain-fed, hot semi-arid ecosystem of western India was conducted. Nine Bael varieties, *viz.,* NB- 5, NB-7, NB-9, CISH-B-1, CISH-B-2, Pant Shivani, Pant Urvashi, Pant Aparna and Pant Sujata (10-year-old) were evaluated Pulp TSS was found highest in 'NB-9' followed by 'Pant Aparna' and 'Pant Shivani' **(Singh *et al.,* 2014)**. The maximum total soluble solids were found in Bael genotype 'VB-14' followed by 'VB-12' and it was minimum in 'VB-4' followed by 'VB-15' **(Rao *et al.,* 2016)**. Fourteen Bael genotypes were evaluated at the Regional Horticulture Research Station, Chapainawabganj, Bangladesh to find out the superior genotypes. The highest TSS (%) was recorded in 'AM 03' and 'AM 04', while the minimum was found in 'AM 13' **(Uddin *et al.,* 2016).** The maximum Total Soluble Solids (33.77 ^{0} Brix) of fruit was recorded in 'Acc.19' of Bael among the different genotypes **(Pavani *et al.,* 2018)**.

2.1.3.3. Phenylalanine ammonia lyase (PAL)

The PAL enzyme acts on the removal of the ammonia group from the aromatic amino acid phenylalanine, transforming it into trans-cinnamic acid, which is precursor of phenolic compounds. Thus, it is important to estimate the activity of this enzyme for the selection of genotypes to be used in breeding programs, since when it is found in greater activity, fruits with greater possibility of defences against pathogens will be produced, being determinant in the resistance of plants to diseases. The level of PAL activity depends on the genotype and also the age and developmental stage, the organ, and even tissue type of the plant **(Camm and Towers, 1973)**. PAL activity is affected by a number of factors including light, temperature, growth regulators, inhibitors of RNA and

protein synthesis, wounding, and mineral nutrition **(Jones, 1984)**. In a study entitled Phenylalanine ammonia-lyase (pal) activity and its relationship to anthocyanin and flavonoid levels in New Zealand-grown apple cultivars found that, wide variation in the level of PAL activity in different apple cultivars (**Lister *et.al.,* 1996).** The highest levels of Phenylalanine ammonia-lyase (pal) activity in the peach genotypes 'Cascata 967', 'Conserva 985', 'Kampai', 'Tropic Snow' and 'Cascata 1055' **Fabiane *et al.,* (2017)**.

2.1.3.4. Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) activity was determined in pulp extract of 'Grand Naine' showed highest activity of enzyme followed by 'Basrai' and 'Galli Solgate' **(Deshmukh *et al.,* 2009)**. Polyphenol oxidase activity in twenty two selected apple cultivars studied and found that, 'Angold', 'Selena' and 'Gold Milenium' showed the highest PPO activity whereas 'Rebella', 'Šampion', 'Topaz', 'Rewena', 'Enterprise' and 'Gerlinde' showed the lowest PPO activity **(Kołodziejczyk *et al*., 2010)**.

2.2. Molecular characterization

The molecular characterization refers use of molecular markers that detect variation at the DNA level, overcome most of the limitations of biochemical and morphological markers. Molecular markers are variants in the DNA sequence, that can be readily detected and whose inheritance can be monitored.The molecular characterization of fruit crops exhibit great significance. Especially when, there is unavailability of systematic data related to morphological characterization and evaluation of the fruit. The conventional breeding is laborious and very lengthy and in fruit crops it takes more than 15 years to develop a variety due to perennial nature and specific edaphic and climatic needs of fruit crops. Fruit species are mostly heterozygous due to high degree of outcrossing. Some of the semi-wild and wild species of underutilized fruit crops are on a way of extinction. Therefore, molecular characterizations are important to overcome all these limitation of conventional fruit breeding. Molecular markers have diverse applications in fruit crop improvement, particularly in the areas of genetic diversity and varietal identification studies, gene tagging, hybrid detection, sex differentiation, disease diagnostics and marker assisted selection.

Classes of molecular markers

1. Hybridization based molecular marker: RFLP.

2. Polymerase chain reaction based marker RAPD, AFLP, SSR, ISSR *etc.*

3. DNA sequence based: ETS, SNP, STS etc.

Table 2.2 : Comparison of most commonly used marker system

Feature	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required (µg)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	High	High	Moderate	Moderate	High
PCR-based	No	Yes	Yes	Yes	Yes
No. of polymorph loci analyzed	1.0-3.0	1.5-50	20-100	1.0-3.0	1.0
Ease of use	Not easy	Easy	Easy	Easy	Easy
Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low

2.2.1. Molecular characterization in Bael

In Bael very limited study had been done in past regarding its molecular characterization. The few study using different molecular maker are included in this paragraph. The RAPD markers are used to study the genetic relationship and genetic diversity among various genotype of Bael and found that, the similarity coefficient is ranged from 0.33-1.00. The 23SS1047, 26SS10C10 and 28SS10C10 primer found to be polymorphic for Bael **(Dabral, 2006).** In another study related to evaluation of genetic variability of superior Bael genotypes collected from different parts of Andaman Islands, India using fruit characters and random amplified polymorphic DNA (RAPD) markers. A total of 476 polymorphic loci were identified with mean value of 39.66 bands per primer and 63.99 per cent polymorphism. Application of unweighted pair

group method using arithmetic average cluster analysis generated three genotypic groups. 'Bael-5' and 'Bael-8' were most similar genotypes whereas 'Bael-7' and 'Bael-1' were extreme divergent (**Nayak *et al.,* 2013**). The analysis of diversity in Bael by citrus based microsatellite set was undertaken because molecular markers are DNA based markers and reveal the genetic diversity which is more universal. Genetic diversity of Bael was measured by using 10 microsatellite markers. A total of 47 alleles were detected in Bael across the 10 loci investigated, all these alleles were polymorphic, thus revealing a level of 100 per cent polymorphism. The number of observed alleles assorted flanked by 4 to 7 with mean 4.7±1.059 alleles at each locus. The experimental no. of alleles intended for every 10 loci gone beyond the effectual no. of alleles that assorted between 1.384 and 3.164 with an average value 1.995±0.11 **(Shrama and Sharma, 2015)**. The genetic diversity analysis of medicinally important horticultural crop Bael using ISSR Markers done by **Mujeeb *et al.* (2017).**

2.2.2. Molecular characterization of other Rutaceae members

About Twenty-four simple sequence repeat (SSR) markers were used to detect molecular polymorphisms among 370 mostly sexually derived *Citrus* accessions from the collection of citrus germplasm maintained at the University of California, Riverside. A total of 275 alleles were detected with an average of 11.5 alleles per locus and an average polymorphism information content of 0.625. Phylogenetic relationships among all citrus accessions and putative non-hybrid *Citrus* accessions were determined by constructing neighbor- joining trees. These separate analyses (distance and model based) both support the hypothesis that there are only a few naturally occurring species of *Citrus* and most other types of *Citru*s arose through various hybridization events between these naturally occurring forms **(Barkley *et al*., 2006)**. The genetic diversity analysis of Iranian citrus varieties using micro satellite (SSR) based markers revealed that, all fifteen Loci assayed in citrus plant possessed a high level of polymorphism, with the number of alleles per Locus ranging from 4 in TAA41 to 12 at CAT01, ATC09, AG14 (an average, 8.27 alleles were detected per Locus). Cluster analysis with SSR markers resulted in 2 cluster groups **(Jannati *et al.,* 2009)**. In a genetic diversity study of 20 Tunisian orange cultivars by using seven simple sequence repeat (SSR) loci, found that in total, 37 alleles and 44 genotypes were scored. The sizes of alleles ranged from 90 to 280 bp. The number of alleles per locus was from 4 to 7, with an

average of 5.28. Polymorphic information content value changed from 0.599 to 0.769 with an average of 0.675. Analysis of the genotypes revealed a heterozygote deficiency across all the genotypes. The observed heterozygosity varied from 0 to 1 (average of 0.671). Cluster analysis showed that three groups could be distinguished and the polymorphism occurred independently of the geographical origin of the studied orange cultivars. The detected SSR genotypes allowed the establishment of an identification key with a discriminating power of 100 per cent. Multivariate analysis and the neighbor-joining phylogenetic tree indicated a narrow genetic base for the orange cultivars **(Mahjbi *et al.,* 2016)**. The Genetic variability and fingerprint profiles of 19 indigenous and exotic mandarin genotypes were determined by using 60 SSR markers. Of the 57 SSR markers amplified, a total of 96 alleles were detected by 39 polymorphic SSR loci and maximum 5 alleles were amplified with an average of 2.46 alleles per primer pair. The CAT01 was the highly informative marker as it revealed maximum number of alleles (5), PIC value (0.75) and genetic diversity (0.79). Twenty six SSRs revealed specific/unique alleles and identified nine genotypes including all the hybrids. Across the genotypes, maximum number of alleles (83) was detected in Daisy hybrid and the percentage of polymorphic marker was maximum (80.32) in Nova hybrid. The markers with low number of alleles were able to differentiate the varieties with specific alleles. The higher average expected heterozygosity (35.6%) with in a mandarin group as compared to the average observed heterozygosity (27.2%) may be explained by selfing, which reduced the proportion of heterozygotes. The genotypes were classified in three clusters. The low observed heterozygosity frequency, PIC value, and number of alleles explained the narrow genetic base in the present set of mandarin genotypes (**Singh *et al.,* 2016)**[b]. Twelve Simple Sequence Repeat (SSR) primer pairs were used to assess the genetic diversity of 62 acid lime landraces. The average genetic similarity level among the 62 accessions were 0.77, ranging from 0.54 to 1.0 and separated five major cluster groups. Total of 33 alleles were detected by eleven primer pairs and size of alleles ranged from 50 to 225. Average polymorphic information content (PIC) value was 0.50, whereas highest 0.75 and lowest 0.18 was observed in CAT01 and GT03 loci, respectively. The results of the study clearly indicated that, SSR markers are highly polymorphic and more informative for the assessment of genetic diversity of acid lime landraces **(Shrestha *et al*., 2012)**. Simple sequence repeat markers (SSRs or

microsatellite markers) in citrus used to evaluate the efficiency of these markers for characterization of sweet orange. The number of alleles ranged from 1 to 4, with a mean of 2 alleles per locus. Four microsatellite loci developed in this study were found to be useful for sweet orange DNA typing. The data obtained from microsatellites loci considered polymorphic will be useful as tools in the selection of zygotic and nucellar plants, identification of seedlings for the cultivars **(Novelli *et al.* 2017)**.

2.2.3. Transferability of SSR markers:

The SSRs from peach used for apricot with 55 % transferability **(Hormaza, 2002)** and for Cherry with 45 per cent transferability **(Dirlewanger *et al.,* 2002)**. The SSRs from apple and Prunus used in pear genetic diversity study and illustrated 45 and 11 per cent transferability **(Yamamoto *et al.,* 2002)**. Pulasan and litchi are from the same family, the transferability study of 12 Litchi simple sequence repeat (SSR) loci to pulasan revealed that, transferability was 58.3% and the percentage of polymorphic SSR markers was 25 per cent (**Sim *et al.,* 2005)**. A set of 12 primer pairs of simple sequence repeats (SSRs) previously developed for litchi has been evaluated for polymorphism in 16 ackee trees from different populations. Seven primer pairs have been found to be transferable, and four have revealed polymorphisms. However, the average number of alleles per locus has dropped from 4.9 for lychee to 3.7 for ackee. Characterization of the four polymorphic markers in 279 individuals belonging to14 different ackee populations from Benin has revealed that the numbers of alleles per locus range from two to 14 with a mean number of 5.8. The observed and expected heterozygosities range between 0.020 to 0.359 and 0.020 to 0.396, respectively **(Ekue *et al.,* 2009)**. The transferability of SSR from pear to different species varied from 58.2 % (apple) to 11.9 % (cherry). Two pear SSR markers, NAUpy43c and NAUpy55k, could distinguish the 20 different apple genotypes thoroughly, and UPGMA cluster analysis grouped them into three groups at the similarity level of 0.56. The high level of polymorphism and good transferability of pear SSRs to Rosaceae species indicate their promise for application to future molecular screening, map construction, and comparative genomic studies among pears and other Rosaceae species **(Fan *et al.,* 2013)**.

2.3. Development of appetizer from diverse genotypes of Bael

2.3.1. Quality analysis of appetizer

2.3.1.1. Effect of genotype on quality analysis

Peach genotype influenced the postharvest quality of canned peaches, significant variation is observed in TSS, tritratable acidity and total sugar of canned peaches **(Kader *et al.,* 1982)**. A study was conducted to evaluate the quality of the leather from five different cultivars of guava. The highest moisture content was observed in leather from Red Fleshed. The highest TSS was observed in 'Allahabad Safeda' and 'Apple Colour'. The leather acidity was affected by cultivars significantly. The maximum mean acidity was observed in leather from 'Allahabad Safeda' and lowest in 'Red Fleshed' **(Jain and Nema, 2007)**. The nectar prepared from guava variety L-49 had highest ascorbic acid, pH and non-reducing sugar **(Choudhary *et al.,* 2008).** Ascorbic acid content in aonla mouth freshener varied from 48.7 to 114.5 and 48.3 to 108.4 mg/100 g in 'Desi' and 'Banarsi' cultivars, respectively. Maximum ascorbic acid was on the preparation day **(Barwal *et al.,* 2010)**. In a study related to effect of cultivars on standardization of guava R.T.S found that, Highest TSS, acidity% and ascorbic acid content was observed in 'Sardar' followed by 'Apple guava' lowest in Red Fleshed guava **(Abhangrao *et al.,* 2017).**

2.3.1.2. Effect of recipe on quality analysis

A study was carried out to develop Bael fruit pulp based intermediate moisture product and found that superior product recorded 14% fibre content and 15.5% moisture content**(Liyanaduragc *et al*., 2007)** . In another study, it was found that treatment T6 (1.5 g spices + 3g citric acid \ kg apple pulp) proved to be the best in terms of quality analysis compare to the other treatment in preparation of value added apple butter **(Elbelazi *et al.,* 2015)**.An experiment entitled "A study on development of herbal food product- Bael (*Aegle marmelos*) fruit toffee" and found that moisture content of Bael fruit toffees were ranged from 8.8 to 13.7% **(Bhatt and Verma 2016)**.Standardization of recipes and acceptability of value added products of aonla pulp revealed that, quality analysis it was evident from the result that all the parameters showed significant variation among all the treatments. Treatment T4 (60% aonla+ 40% guava+ 125g sugar) scored maximum values for acidity. Treatment T9 (40% aonla pulp+ 60% guava pulp+ 500g sugar) scored maximum values of TSS **(Bisen *et al.,* 2017)**.

2.3.1.3. Interaction effect of genotype and recipe on quality analysis

On the basis of investigation on effect of various recipes and cultivars on preparation of guava RTS, it was illustrated that that the various parameters of quality was observed better with 11% guava pulp and 110 g of sugar per liter of RTS in both the cultivars *i.e*. Pink guava and Allahabad safeda. **(Jhade, 2013)**. In the evaluation of mango cultivars and recipes for preparation of RTS beverage, it is conducted that the various parameters of quality were observed better with 0.75g citric acid and 140 g. of sugar per liter of RTS with the cultivar Totapuri **(Chhigarha, 2015)**. The interaction effect of recipes and cultivars on standardization of guava R.T.S found to be siginicant for TSS, acidity per cent and ascorbic acid. R3V1 (10%pulp, 12% T.S.S, 0.3% Acidity +L-49) shows best combination medium content of TSS and acidity percentage **(Abhangrao *et al.,* 2017)**.

2.3.2. Sensory analysis of appetizer

2.3.2.1. Effect of genotype on sensory analysis

The organoleptic quality of the leather prepared from Allahabad Safeda was found to be the best among all the cultivars followed by 'Sardar' **(Jain and Nema, 2007)**.The nectar prepared from variety 'Sardar' had a higher sensory score followed by 'Allahabad Safeda', 'Apple Colour' and 'R-72' in fresh samples (**Choudhary *et al.,* 2008)**.

2.3.2.2. Effect of recipe on sensory analysis

The study was carried out to develop fibre enriched Bael fruit pulp based intermediate moisture food using potato, sugar and salt as ingredients, with the aim of improving the palatability and increasing the utilization of the fruit and to increase the popularity of Bael fruit among people. Three treatments included the ratios of Bael: Potato: Sugar; 3:2:1, 2:3:1 and 1:1:0 respectively. Sensory evaluation was done using 30 semi trained panelists. According to their preference they selected 2:3:1 (Bael: Potato: Sugar) ratio as the best ingredients combination **(Liyanaduragc *et al*., 2007)**. Mouth freshener prepared with 50% dehydrated aonla pulp, 15% fennel, 10% arecanut and 20% sugar was adjudged best on the basis of overall sensory acceptability attributes during storage for 180 days, irrespective of cultivars. The mouth freshener developed had herbal formulation having potential health benefits **(Barwal *et al.,***

2010). The Sensory characteristic of Bael vermouth illustrated that, the Bael vermouth with treatment 2% spice level was more acceptable organoleptically than 3.5 and 5% spice levels **(Chauhan *et al.,* 2016)**. The results revealed that treatment T9 (40% aonla+ 60% guava+ 500g sugar) showed maximum rating for colour, texture, taste, and overall acceptability during initial i.e. 0 day of storage period while elasticity was found maximum in the treatment T12 (40% aonla pulp+ 60% guava pulp+ 125 g sugar) and flavour was found maximum in the treatments T10 (40% aonla pulp+ 60% guava pulp+ 375g sugar) **(Bisen *et al.,* 2017)**. The sensory analysis of value added bakery products using Bael revealed that, the treatments with added Bael powder, B2 (10 per cent Bael powder) scored the highest **,** whereas the treatments added Bael powder, B4 (20 per cent Bael powder) received the least scores for all the sensoary parameters **(Kaur and Kochhar , 2017)**. The best recipe of Bael candy was found 2 per cent alum concentration and the standardized processed product was stored at room (25-37°C) and refrigerated temperatures (8-10°C) up to 8 and 12 months and with organoleptic score 4.66 and 4.94 was found best among all treatments of local and NB-5 cultivar respectively **(Singh *et al.,* 2017)**. The study on development of value added products from Bael fruit showed that, Bael juice with recipe jiggery and lime, Bael jam and toffee of combination of equal amount of sugar organoleptically scored higher and accepted. **(Ullikashi *et al.,* 2017)**. In an investigation entitled "physico-chemical properties of value added apple butter ", revealed that among the different treatment T4 (1.5g spices + 2g citric acid per kg apple pulp) proved to be best in terms of sensory score. The Development and organoleptic evaluation of mixed fruit leather from Bael and Aonla pulp resulted that , the sensory level ranked the best product at 50% level of aonla pulp and 50% of Bael pulp with respect to colour, flavour, texture and overall acceptability **(Uttarwar *et al.,* 2018)** .

2.3.2.3. Interaction effect of genotype and recipe on sensory analysis

The physico -chemical and sensory properties of four grape juice blends prepared from three selected grape cultivars, 'Castel 19637', 'Lucie Kulman', 'Sovereign Coronation' and water were evaluated on the selection for a non-alcoholic wine-like beverage. A descriptive sensory study was conducted using 18 trained panelists to assess the colour, sweetness, astringency, viscosity and overall acceptability of the beverages. Overall, considering the sensory attributes the blend of

'Castel 19637': water (3:2, v:v) was found to have more potential to be used in developing a wine-like functional beverage. 'Castel 19637', which was originally developed as a red wine grape cultivar, can be recommended as a suitable grape cultivar for developing a functional beverage **(Ratnasooriya *et al.,* 2012)** .An investigation entitled "Effect of various recipes and cultivars on preparation of guava RTS" was carried out in the Post Harvest laboratory, JNKVV (Jabalpur) , revealed that, overall acceptability was observed better with 11% guava pulp and 110 g. of sugar per liter of RTS in both the cultivars *i.e.* 'Pink guava' and 'Allahabad safeda' **(Jhade, 2013)**. The overall acceptability weas observed better with 0.75gm citric acid and 140 gm. of sugar per liter of RTS with the cultivar Totapuri **(Chhigarha, 2015)** .

Materials and Methods

Chapter 3 MATERIALS AND METHODS

The study entitled "Biochemical and molecular characterization of diverse genotype of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties" was carried out at the Horticulture Research Centre, Pattharchatta, G. B. Pant University of Agriculture and Technology, Pantnagar, District, U. S. Nagar, Uttarakhand during the year 2016-18. The biochemical characterization and product preparation were performed in the Post Harvest Laboratory of the Department of Horticulture. The molecular characterization was performed in the Biosafety and Molecular Diagnostics Laboratory of Department of Molecular Biology and Genetic Engineering (MBGE), CBSH, GBPUA&T, Pantnagar. The details of materials and methods followed during the course of investigation have been described in this chapter.

3.1. Experimental Site and Location

The experimental site Horticultural Research Centre, Patharchatta is located 8 km away from the main campus of G. B. Pant University of Agriculture & Technology, between 29.02 ºN latitude and 79.30 º E longitudes and at altitude of 243.84 meters above mean sea level.

3.2. Climatic Conditions

The climatic condition of experimental site is humid subtropical. The summer is dry and hot, and the winter is cold and the rainy season is with heavy rainfall (1400mm). Usually the onset of Monsoon occurs in the third week of June and continues in appreciable amount up to mid of September. Frost can be expected from the last week of December to first week of February. Occasionally light rains occurs during winter. Weather data for period of investigation have been presented in appendix I and II.

3.3. Soil Conditions

The soil of the experimental site has been classified as series VI Pattharchatta sandy loam under the order mollisols (Deshpande *et al.,* 1971). Soil is dark coloured, imperfectly drained with moderate to high organic matter content developed in loamy alluvial sediments averaging 0.6 to 1.0 meter thick over loamy sand, sand or gravel. The soil has high cation exchange capacity and also contains about 90 per cent saturation. The physical and chemical charaters of patharchatta sandy loam are presented in Table 3.1 and 3.2.

Table 3.1: Physical characters of Patharchatta sandy loam soil

S. No.	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Bulk Density (g/cm^3)	Moisture content (%)		Hydraulic conductivity (cm/hr)
						13 atm.	15 atm	
1.	0-20	53.2	35.5	11.2	1.58	20.3	5.2	8.46
2.	20-38	53.6	34.2	12.2	1.54	19.1	5.3	6.25
3.	38-48	60.9	27.6	11.5	1.52	16.4	4.9	6.43
4.	48-74	69.5	21.8	8.7	1.54	12.6	4.2	9.21
5.	74-107	73.2	17.3	9.6	1.49	10.5	4.0	10.34
6.	107-129	73.6	17.3	9.2	1.50	11.0	4.2	11.38

Table 3.2: Chemical characters of Patharchatta sandy loam soil

S. No.	Depth (cm)	Organic matter (%)	pH	$CaCO_3$ Equivalent	CE (me/100 g)	Exchangeable cations (me/100 g)				Base Saturation (%)
						Ca	Mg	K	Na	
1.	0-20	1.9	6.4	0	9.8	6.7	2.3	0.21	0.09	95
2.	20-38	1.1	6.1	0	8.6	4.9	1.8	0.14	0.09	80
3.	38-48	0.9	6.2	0	7.5	4.0	1.6	0.14	0.10	80
4.	48-74	0.6	6.2	0	6.2	3.3	1.5	0.12	0.05	7
5.	74-107	0.5	6.2	0	5.8	3.1	1.4	0.10	0.04	80
6.	107-129	0.4	6.4	0	6.6	3.7	1.6	0.04	0.04	82

3.4. Experiment materials

The experiment was carried out on 24 genotypes of Bael at Horticultural Research Centre, Patharchatta. The five one year old budded plants of each genotypes were planted at the distances of 5.0 metres in square system during the year 1987 at H.R.C., Patharchatta. Three healthy plants of each genotypes selected for the experiment were almost uniform in growth and vigour and maintained under similar cultural operations.

3.5. Treatment and experiment design

The 24 genotypes Bael were considered as treatment in the present study. The experiment was laid out in Completely Randomized Design (CRD) with three replications and 18 treatments for the biochemical analysis. For biochemical characterization fruits of 18 genotypes were harvested during March. The 24 genotypes Bael is used for molecular characterization. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with three replications and 66 treatment combinations for the appetizer development. The details of treatment and symbols allotted to them are given below.

Table 3.3: Treatment details for biochemical characterization

T_1	:	Pant Bael-1	T_{10}	:	Faizabad Local
T_2	:	Pant Bael-2	T_{11}	:	Pant Aparna
T_3	:	Pant Bael-3	T_{12}	:	Pant Bael-10
T_4	:	Pant Bael-4	T_{13}	:	Pant Sujata
T_5	:	Pant Shivani	T_{14}	:	Pant Bael-13
T_6	:	Pant Urvashi	T_{15}	:	Pant Bael-14
T_7	:	Haldi Nurmohamd	T_{16}	:	Pant Bael- 15
T_8	:	Patharchatta-1	T_{17}	:	Pant Bael -16
T_9	:	Faizabad No.9	T_{18}	:	Gonda No.2

Table 3.4: Treatment details for molecular characterization

L_1	:	Pant Bael-1	L_{13}	:	Pant Aparna
L_2	:	Pant Bael-2	L_{14}	:	Pant Bael 10
L_3	:	Pant Bael-3	L_{15}	:	Pant Bael 11
L_4	:	Pant Bael-4	L_{16}	:	Pant Sujata
L_5	:	Pant Shivani	L_{17}	:	Pant Bael-13
L_6	:	Pant Urvashi	L_{18}	:	Pant Bael-14
L_7	:	Haldi Nurmohamd	L_{19}	:	Pant Bael 15
L_8	:	Gonda Abdullahpur	L_{20}	:	Pant Bael 16
L_9	:	Patharchatta-1	L_{21}	:	Gonda No.2
L_{10}	:	Faizabad No.1	L_{22}	:	Pant Vishal
L_{11}	:	Faizabad No.9	L_{23}	:	NB-9
L_{12}	:	Faizabad Local	L_{24}	:	NB-7

Treatment details for preparation of appetizer

Factor A	:	**Genotype**
A_1	:	Pant Bael-1
A_2	:	Pant Bael-2
A_3	:	Pant Bael-3
A_4	:	Pant Bael-4
A_5	:	Pant Shivani
A_6	:	Pant Aparna
A_7	:	Pant Bael-10
A_8	:	Pant Bael-14
A_9	:	Pant Bael -15
A_{10}	:	Pant Bael -16
A_{11}	:	Gonda No.2

Factor B: Ingredients

B_1 : 100% Bael powder,

B_2 : 96% Bael powder + 3% Black salt + 1% Black pepper,

B_3 : 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,

B_4 : 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**

B_5 : 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**

B_6 : 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

Treatment combinations

A_1B_1	A_2B_1	A_3B_1	A_4B_1	A_5B_1	A_6B_1	A_7B_1	A_8B_1	A_9B_1	$A_{10}B_1$	$A_{11}B_1$
A_1B_2	A_2B_2	A_3B_2	A_4B_2	A_5B_2	A_6B_2	A_7B_2	A_8B_2	A_9B_2	$A_{10}B_2$	$A_{11}B_2$
A_1B_3	A_2B_3	A_3B_3	A_4B_3	A_5B_3	A_6B_3	A_7B_3	A_8B_3	A_9B_3	$A_{10}B_3$	$A_{11}B_3$
A_1B_4	A_2B_4	A_3B_4	A_4B_4	A_5B_4	A_6B_4	A_7B_4	A_8B_4	A_9B_4	$A_{10}B_4$	$A_{11}B_4$
A_1B_5	A_2B_5	A_3B_5	A_4B_5	A_5B_5	A_6B_5	A_7B_5	A_8B_5	A_9B_5	$A_{10}B_5$	$A_{11}B_5$
A_1B_6	A_2B_6	A_3B_6	A_4B_6	A_5B_6	A_6B_6	A_7B_6	A_8B_6	A_9B_6	$A_{10}B_6$	$A_{11}B_6$

3.6. Experimental Detail

3.6.1. Selection of trees

Twenty nine year old healthy and uniform tree of 24 genotypes of Bael were selected for present study. Three tree of each genotype were selected randomly. Thus total number of trees used in this experiment were 72. The uniform cultural treatment was given to those trees during the course of investigation.

3.6.2. Observation

Observations based on biochemical analysis of fruits of 18 genotypes were recorded during 2016-17. Observation based on molecular characterization of 24 genotypes and quality analysis of appetizer were recorded during 2017-18. The details of observation and methods applied are described as below.

3.6.2.1. Biochemical analysis

3.6.2.1.1. Ascorbic acid

The Ascorbic acid content of fruits were estimated by using 2, 6-Dichlorophenol-indophenol visual titration method (Ranganna, 1986). Ascorbic acid in terms of mg per 100g pulp weight was calculated by using the following formula:

$$Ascorbic\ acid\ mg/\ 100g = \frac{Dye\ factor \times titre\ reading \times dilution \times 100}{Weight \times Volume\ of\ a\ sample}$$

3.6.2.1.2. Marmelosin

Fruit pulp was dried to make fine powder. About 10 g accurately weighed powdered was extracted with methanol in a Soxhlet apparatus for 6 hour at temperature 80° C (Shinde *et al.,* 2014). The extract was filtered, transfer to 100 ml volumetric flask and make up the volume with methanol. HPLC analysis was performed with a Agilent 1120 LC Germany system consisting of an Double-beam photometer, Deuterium lamp, wave length ranges from 190-600nm. Gradient pump includes an integrated dual-channel degasser, Manual injector with 20 µL loop. Compounds were separated on a 250 mm×4.6 mm i.d., 5-µm particle, C-18 column. The 55:45 (%, v/v) methanol-water containing 0.1 % acetic acid as isocratic mobile phase at a flow rate of 1.0 ml min^{-1}. The injection volume was 20 µL and the detection wavelength was 300 nm. HPLC was performed at ambient temperature and data were analyzed on a computer equipped with EZChrom Elite software.

3.6.2.1.3. Total Phenol content

Total phenols content present in fruits were determined by Folin Ciocalteu reagent (Mcdonald *et al.,* 2001).A diluted plant extract (0.5mL of 1:10g/mL-1) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4mL, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250mg/L solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg GAE/g of dry mass), which is a common reference compound.

3.6.2.1.4. Pectin content

Pectin content was measured by method described by Mazumder and Majumder (2003). Known amount of fruit pulp sample by weight was taken. This is boiled with 0.05 *N* hydrochloric acid (Reagent-A) at a temperature of 80-90^{o}C for 2 hours to extract the pectic substances. After extraction, the mixture is cooled to room temperature. It is then filtered (Whatman No. 4). The extraction was repeated for maximum recovery of the pectin.The filtrate was made upto a known volume with distilled water. An aliquot from the above extract (50 ml) was taken in a conical flask and it was neutralized with 1N sodium hydroxide solution (Reagent-B). The excess amount of sodium hydroxide solution was added to it with thorough mixing till the mixture becomes slightly alkaline. The solution was allowed to stand overnight.Then 10 ml of 1N acetic acid (Reagent-C) was added. After 2 minutes, 5ml of 1N calcium chloride solution (Reagent-D) was mixed with constant stirring. It was left for an hour to precipitate the calcium pectate. The solution was then filtered through a dried and pre-weighed filter paper (Whatman No. 4). The precipitate was washed repeatedly with boiling water to eliminate chloride ions present in the precipitate. The filter paper containing calcium pectate was dried at 50-60 ^{o}C and weighed. The amount of pectin content (as calcium pectate) present in the samples was calculated by the following formula.

$$\text{Pectin content (\%)} = \frac{d \times c}{b \times a} \times 100$$

Where,

a = Weight of sample

b = Volume of aliquot taken for estimation

c = Volume made with distilled water

d = Weight of calcium pectate.

3.6.2.1.5. Riboflavin

The riboflavin was estimated by the method of AOAC (1980). The flurometric procedure for the determination of riboflavin depends upon the extraction of the vitamin with dilute acid, filtration, oxidation of the filtrate with potassium permanganate and hydrogen peroxide to destroy interfering pigments and measurement

of the fluorescence of riboflavin. The riboflavin measured in terms of the difference between the fluorescence before and after chemical reduction. The results were expressed as mg/100g.

3.6.2.1.6. Total carotenoids

One gram of sample was weighed and grinded with acetone using acid and alkali washed sand in a pestle and mortar. The extract is decanted into a conical flask. The extraction was continued till the residue was colourless. The acetone extract was transferred to a separating funnel containing 10-15 ml of petroleum ether and mixed gently. After addition of 25 ml of 5 per cent sodium sulphate, the solution was shaked and kept for sometimes. The separated yellow colour pigment was transferred into the petroleum ether later. The layer was collected in a volumetric flask and acetone layer containing Na_2SO_4 was separated until the colour gets transferred into the petroleum ether. The colour intensity was measured at 452 nm by using spectrophotometer and the total carotenoid content was calculated by the following formula.

$$Total\ Carotenoids\ mg/100g = \frac{3.857 \times O.D \times Volume\ made\ up \times 100}{weight\ of\ sample \times 100}$$

3.6.2.1.7. Total falvonoids

Total flavonoids were measured by procedure given by Dewanto *et al.* (2002). An aliquot of diluted sample or standard solution of catechin was added to 75 mL of $NaNO_2$ solution (5%) and mixed for 6 min before addition of 0.15 mL $AlCl_3$(10%). After 5 min, 0.5 mL of NaOH were added. The final volume was adjusted to 2.5 mL with distilled water and mixed thoroughly. Absorbance was determined at 510 nm against a blank. The total flavonoid content was expressed as milligrams of catechin per gram of dry weight (mg CE/g DW) against the calibration curve of catechin, from 0 to 100µg/mL.

3.6.2.1.8. Superoxidase dismutase (SOD)

The activity of SOD in fruit skin or pulp tissue was assayed spectrophotometrically by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT), as reported by Abassi *et al.,* (1998) with slight modification.

3.6.2.1.9. Catalase (CAT)

CAT activity in fruit skin and pulp was assayed by using method of Luck (1965). The reaction was carried out using two buffer solutions. The optical density at 240 nm of the solution was recorded at 45 and 60 seconds starting from the time when the extract was added to the cuvettes, using a UV-vis Spectrophotometer The difference in optical density between the 45 and 60 seconds readings was used to calculate CAT activity as reported by Luck (1965).

3.6.2.1.10. Peroxidase (POD)

The method proposed by Reddy *et al.,* (1995) was adopted for assaying the activity of peroxidase. In the presence of the hydrogen donor pyrogallol, peroxidase converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm.

3.6.2.1.11. DPPH radical scavenging (%)

The hydrogen atom or electron donation capacity of the extracts was measured as a decrease in absorbance of DPPH. The scavenging ability were determined based on the method given by (Yamaguchi, 1998). The reaction between antioxidant compounds with the stable DPPH radical will caused reduction in absorbance and decolourisation of DPPH to light yellow14. To 1.5 mL of methanolic DPPH solution (0.1 mM) was introduced varying concentrations of the extract in methanol [0.1 mg/mL (100 µgm/mL) – 5.0 mg/mL (5000 µgm/mL)]. A control sample was prepared without extract containing DPPH solution in a appropriate volume. Methanol was used as a blank. The mixtures obtained were shaken well and left for 20 minutes at room temperature and the absorbance of the resulting solutions were taken at 517 nm against a blank in UV visible spectrophotometer. The radical scavenging activity was measured as a decrease in the absorbance of DPPH.

$$\text{Per cent scavenging activity} = \frac{\text{1- Abs sample} \times 100}{\text{Abs Control}}$$

3.6.2.1.12. Total soluble solids (TSS)

The juice of Bael was squeezed by hand and the total soluble solids of fruit were measured by using digital hand refractrometer (Atazo, Japan) at room temperature and results were expressed in terms of degree Brix (°B).

3.6.2.1.13. Phenylalanine ammonia lyase (PAL)

PAL activity in the buffer supernatant was determined by the production of cinnamate during 1 h at 30C, as measured by the absorbance change at 290 nm (Zucker, 1965). The assay mixture contained 15 mol L-phenylalanine, 30 mM sodium borate buffer (pH 8.8), and 0.2 to 0.5 ml buffer supernatant, depending on the PAL activity level, in a total volume of 3.0 ml. The substrate was added after 10 min of preincubation and the reactions stopped with 0.1 ml 6 N HCl. Assays were performed in triplicate.

3.6.2.1.14. Polyphenol oxidase (PPO)

A spectrophotometric method was used to determine PPO activity on basis of the initial rate of the absorbance increase at 420nm (Rocha and Moris, 2001). Enzyme activity was assayed in 3 ml of reaction mixture consisting of 0.1 ml substrate (0.1 M 4-methylcatechol, catechol or L-Dopa) and 0.1 ml enzyme preparation in 0.1 M phosphate buffer (pH 6.5). The activity of PPO was determined by measuring the absorbance at 420 nm using an UV-Vis spectrometer.

3.6.2.2. Molecular characterization

3.6.2.2.1. Collection of experimental Tissues

(a) Fresh and young leaves were plucked from individual tree of all 24 genotypes and wrapped in aluminum foil.

(b) Samples were placed in an ice box and brought to laboratory from Horticultural Research Centre, Pattharchatta.

(c) Two grams of fresh sample were weighed with electronic balance and placed in -20 ^{o}C refrigerator.

3.6.2.2.2. Isolation of genomic DNA

The method described by Porbeski *et al.* (1997) with slight modification was followed for extraction of genomic DNA. The steps followed are as under.

(a) Using electronic balance 2.0 g of alcohol sterilized leaf material was weighed for DNA extraction.

(b) Pre-weighed leaf material was grind in liquid nitrogen to fine powder using pre-chilled pestle and mortar.

(c) The powder was transferred to 50 ml oak ridges tubes containing 20 ml of pre-warmed extraction buffer. Spatula was used to dispense the material completely.

(d) Samples were incubated in water bath at 65^{o}C for 50 minutes (cap should be slightly loose). Mix it intermittently at almost 10 minutes interval.

(e) After taking tubes out from water bath, it was allowed to come at room temperature and 10 ml of (double amount of sample) chloroform: isoamyl alcohol (24:1) was added and mixed by inversion, swirl in the shape of 8 for approximately 15 minutes to emulsify.

(f) Centrifugation was done at 15,000 rpm at room temperature for 15 minutes.

(g) This above step was repeated 3 times until the solution becomes transparent.

(h) Aqueous phase was removed with a wide bore pipette and transferred to a clean tube, double volume of chilled isopropanol was added and mixed by quick gentle inversion and finally the tubes were kept overnight at -20^{o}C.

(i) The samples were centrifuged for 10 min at 5,000 rpm, 4^{o}C. The supernatant was poured without disturbing the DNA pellet at the bottom of the tubes.

(j) The DNA pellet was washed with 70% ethanol.

(k) The pellet was air dried and dissolved in 500 µl TE buffer. It was stored at -20^{o}C.

The list of buffers and stock solution are given in appendix-V.

3.6.2.2.3. DNA purification:

RNase A treatment

Ribonuclease A is an endonuclease enzyme, having the capacity to digest RNA. DNase free RNase powder, supplied by Genie Pvt. Ltd., Bangalore was used. This RNase was supplied as a powder and dissolves easily in water. Preparation one ml sterile de-ionized water (zero conductivity) was added to the vial containing powder. Aliquots of 50µ l were made in sterile eppendorf tubes. The tubes were kept in boiling water at 100^{o}C for 15 minutes; Eppendorf tubes were covered with a Paraffin film and stored at -20^{o}C. RNase treatment procedure.

1. 10µl RNase A (to a final concentration of 50µg/µl) was added to the eppendorf tube containing 200µl of extracted DNA and mixed by swirling the tubes.

2. These tubes were then incubated for 30 minutes at 37°C in dry bath.
3. Equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added in the tube and mixed by inverting the tube gently.
4. The content was centrifuged at 10,000 rpm for 12 min at 4 °C.
5. The aqueous phase was transferred into a fresh eppendorf tube and and 1/10th volume of ice cold 3M sodium acetate (pH 5.2) and double volume of ethanol was added and the mixture was kept at -20°C overnight.
6. The tube was then centrifuged at 10,000 rpm at 10°C for 15min to precipitate the DNA.
7. Supernatant was removed using a micropipette and pellet was washed with 70% ethanol and dried completely.
8. DNA pellet was redissolved in minimum amount of TE buffer.
9. The quantitative estimation was done spectrophotometrically. Quantification is necessary to carry out further dilution of the DNA sample of PCR analysis.

3.6.2.2.4. Qualitative analysis "Agarose gel electrophoresis"

Agarose gel electrophoresis is the standard method used to check the quality of DNA fragments. The technique is simple, rapid to perform and capable of resolving fragments of DNA. For resolving the DNA fragments on gel the DNA samples were loaded with loading dye in 5:1 ratio. The DNA was subjected to electrophoresis in 0.5 X TBE buffers.

The stock solution was used for Agarose gel Electrophoresis is given in appendix-V.

3.6.2.2.5. Quantification of Genomic DNA by U.V. Spectrophotometer

DNA concentration was measured by using UV spectrophotometer (ELICO Ltd.), Blank was set against TE buffer. The OD was measured at 260 nm for estimating the concentration of DNA. The concentration is related to OD by the following equation:

$$DNA\ Conc.(\mu g/\mu l) = \frac{O.D.260 \times 50 \times Dilution\ factor}{1000}$$

Optical density, O.D. was recorded at 260 and 280 nm to calculate the ratio OD260/OD280. The ratio gives the amount of RNA or protein in the preparation. A

ratio of 1.8 is optimum for best DNA preparation. A value of the OD260/OD280 ratio below 1.8 indicates the presence of proteins in the preparation and a value above 1.8 indicates that the sample has a lot of RNA.

3.6.2.2.6. PCR Amplification

The Polymerase Chain Reaction (PCR) is a powerful, extremely sensitive technique based on the enzymatic amplification of DNA fragments that are flanked by oligo-nucleotide primer(s) hybridizing to opposite strands of the target sequence. The PCR involves three basic steps which constitute a single cycle: (i) Denaturation of the target DNA at 92-94 ^{o}C. (ii) Annealing of the primers to the template DNA. (iii) Primer extension by addition of nucleotides at 3‟ end of the primers by the enzyme DNA polymerase.

3.6.2.2.7. PCR Ingredients

i. Template DNA

The Genomic DNA isolated from 24 genotypes of Bael was diluted to a final concentration of about 100ng/µl prior to use.

ii. Design and synthesis of the primers

The most essential requirement of PCR is the availability of short oligonucleotides called primers having sequences complementary to either ends of the target DNA segment called template DNA to be synthesized. The primers used in the study were obtained from G Biosciences. The primers sequences used for SSR analysis are shown in Table 3.7.

iii. *Taq* DNA polymerase

Taq DNA polymerase (5.0 U/µl) from G-Biosciences was used in each reaction mixture to a final concentration of 1.5U per PCR reaction.

iv. dNTP's

The dNTPs used in the reaction were obtained from Thermo Scientific. 1.0 µl of each dNTPs (10 mM) was used in reaction mixture.

v. Assay buffer (10X)

Assay buffer (10X) from G-Biosciences contained 100mM Tris-HCl (pH8.8), 500mM potassium chloride, 1% Triton® X-100, 16mM MgCl2 was used to a final concentration of 1X in each reaction mixture.

vi. Protocol for primer dilution

(a). Primers were received in lyophilized form. The vials were short spin to collect the lyophilized powder at bottom of vials.

(b). Desired amount of molecular grade water was added .this operation was carried out in laminar air flow chamber.

(c). Gentle tapping was done followed by keeping at 4^{o}C for 15 minutes.

(d). Short spinning, followed by gentle inversion was performed. Then the primers were kept at 4^{o}C for 15 minutes.

(e). Again short spinning was performed and primers were stored at -20^{o}C for further use.

3.6.2.2.8. Polymerase chain reaction (PCR) reaction set up

Polymerase chain reaction PCR reaction set for 25 µl reaction has been shown in table 3.2. In the first step all PCR tubes were labelled properly then 1 µl of genomic DNA of concentarion 100ng/µl was added in each PCR tube. A master mix of Molecular Biology Grade water, 10X or 5X Taq buffer (whichever is supplied), dNTP, forward and reverse primers and *Taq* DNA polymerase was prepared to reduce pipetting error. The master mix was then redistributed in each PCR tube. The content was gently mixed in microspin.

Table 3.5: Concentration of reaction mixture in PCR amplification

Components	**Final Concentration**
1. DNA template	100ng
2. dNTP mix (10 mM mix)	10mM (2.5 mM each dNTP)
3. *Taq* DNA polymerase (5U/ µl)	1.25U
4. Reaction buffer (10X)	1X
5. Primer Forward (50ng/ µl)	10pM
6. Primer Reverse (50ng/ µl)	10pM
7. Molecular grade water	To make up the final volume to 25 µl

3.6.2.2.9. Optimization of PCR for SSRs Primers

Optimization of annealing temperature of a primer is an important task. Melting temperature (T_m) of the primer decides proper binding of the primer with the template DNA. If Tm is higher than the optimum one the primer fails to remain bound with the template for that much time by which the *Taq* polymerase comes and starts extending the primer. On the other hand, a T_m lower than the optimum one facilitates non specific binding of the primer with template DNA and hence results in non specific amplification. Annealing temperature was selected to be 5°C less than the theoretical T_m. Therefore, touch down PCR cycle is used to increasing specificity of PCR reactions. Touchdown PCR uses a cycling program where the annealing temperature is gradually reduced (e.g. 1-2°C /every second cycle). The initial annealing temperature should be several degrees above the estimated T_m of the primers. The annealing temperature is then gradually decreased until it reaches the calculated annealing temperature of the primers or some degrees below. Amplification is then continued using this annealing temperature.

Table 3.6: Thermal cycling conditions (programme was set up for the PCR amplification)

Phases	Temperature	Duration	Number of cycle
Initial Denaturation	94 °C	5 mint	1cycle
Denaturation	94 °C	1 mint	20cycle
Annealing	65-47°C	30 sec	
Extension	72 °C	2min	
Denaturation	94 °C	20 sec	20cycle
Annealing	55°C	50 sec	
Extension	72 °C	50sec	
Final Extension	72 °C	5mint	1cycle
Final hold	4 °C	Forever	Take out samples

3.6.2.2.10. Genic Simple sequence repeats (SSRs) markers:

A set of fourty (40) citrus based SSRs primer pairs were employed for PCR amplification shown in Table 3.7.

Table 3.7: Detail of the primer code and their sequence used under study

S.No.	Primer name	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
1	AC01	TTTGACATCAACATAAAACAAGAAA	TTTTAAAATCCCTGACCAGA
2	AG14	AAAGGGAAAGCCCTAATCTCA	CTTCCTCTTGCGGAGTGTTC
3	ATC09	TTCCTTATGTAATTGCTCTTTG	TGTGAGTGTTTGTGCGTGTG
4	CAC15	TAAATCTCCACTCTGCAAAAGC	GATAGGAAGCGTCGTAGACCC
5	CAC23	ATCACAATTACTAGCAGCGCC	TTGCCATTGTAGCATGTTGG
6	CAC33	GGTGATGCTGCTACTGATGC	CAATTGTGAATTTGTGATTCCG
7	CAC39	AGAAGCCATCTCTTCTGCTGC	AATTCAGTCCCATTCCATTCC
8	CAT01	GCTTTCGATCCCTCCACATA	GATCCCTACAATCCTTGGTCC
9	CAG01	AACACTCGCACCAAATCCTC	TAAATGGCAACCCCAGCTTTG
10	CAGG9	AATGCTGAAGATAATCCGCG	TGCCTTGCTCTCCACTCC
11	CCSM13	CTAGAGCCGAATTCACC	AACAGCTACCAAGACACC
12	CCSM17	ACATGGACAGGACAACTAAG	GTTATGATACGTCTGTGTCC
13	CCSM18	GTGATTGCTGGTGTCGTT	AACAGTTGATGAAGAGGAAG
14	CCSM 146	TGGTTAGAAGGTGAACAG	ACATAGAGGTTTGCTTATC
15	CCSM147	GCTATGTTATGATACGTCTG	AGACTCACGTAACCTACTTC
16	CT02	ACGGTGCGTTTTGAGGTAAG	TGACTGTTGGATTTGGGATG
17	CTT01	TCAGACATTGAGTTGCTCG	TAACCACTTAGGCTTCGGCA
18	CCT01	TCAACACCTCGAACAGAAGG	CCCACATGCTAGCACAAAGA
19	CT19	CGCCAAGCTTACCACTCACTAC	GCCACGATTTGTAGGGGATAG
20	CT21	CGAACTCATTAAAAGCCGAAAC	CAACAACCACCACTCTCACG
21	CY01	CGTCTTCCTTCCTTACTT	ATCGGTGAAAATAGCAAC
22	CY05	ACCAAATCACTGAACAAAT	ACATGAGGGACCTTCTTAG
23	CY07	ATAATCAAACTCCCTCGTG	TACAAAATTGGACAGCAA
24	CY13	TGTCGCTCATCATTAGGT	CCAATTCATTTCAAACCC
25	CY19	CGCATTGAAAGTCTGTGGT	ATCTGAAGGCTTCTGTGGC
26	CY23	GTGAATGAAAGAACCCAT	CAAAGCGACTGACAACTA
27	CY37	CCAATACCCAGTTCCAAG	CTACCTCTTCCCTTTTCT
28	CY40	ACAGCGAGATCATTGAGT	TATCGTTATTTACGTGGG
29	CY48	CTGAGGTGGGACTGGTGT	CATCTCGCAGAAAGTAAAA
30	CY51	ACAAAAGCACAAAAGGCCAAA	AGATGAATGCGTGTAGTAAGC
31	GT03	GCCTTCTTGATTTACCGGAC	TGCTCCGAACTTCATCATTG
32	SCM02	GAATGGCTTAGATGACAAA	ATTCACAAACGAAACACT
33	SCM05	CAGCTACTATCAGAAAAATAATCAG	GCACAAAGAGAAAAAGGC
34	TAA1	GACAACATCAACAACAGCAAGAGC	AAGAAGAAGAGCCCCCATTAGC
35	TAA3	AGAGAAGAAACATTTGCGGAGC	GAGATGGGACTTGGTTCACACG
36	TAA15	GAAAGGGTTACTTGACCAGGC	CTTCCCAGCTGCACAAGC
37	TAA27	GGATGAAAAATGCTCAAAATG	TAGTACCCACAGGGAAGAGAGC
38	TAA45	GCACCTTTTATACCTGACTCGG	TTCAGCATTTGAGTTGGTTACG
39	TAA41	AGGTCTACATTGGCATTGTC	ACATGCAGTGCTATAATGAAGT
40	TC-26	CTTCCTCTTGCGGAGTGTTC	GAGGGAAAGCCCTAATCTCA

3.6.2.2.11. Standardization of PCR Conditions There are number of variables in a PCR which have to be optimized in order to give target amplification. These parameters are:

(1) Denaturation temperature and time

(2) Annealing temperature and time

(3) Amount of template DNA and primer to be taken

(4) Concentration of $MgCl_2$ in the assay buffer

(5) The number of cycles to be performed

3.6.2.3. Quality analysis of appetizer

3.6.2.3.1. Reducing sugars

The reducing sugar was estimated by the method of AOAC (1980). The extract was taken and titrated against 10 ml of mixed Fehling solution A and B using methylene blue as indicator. The results were expressed as per cent of reducing sugar.

3.6.2.3.2. Total sugars

Total sugar was estimated by the standard method of AOAC (1980). The sugar extract was hydrolyzed with concentrated hydrochloric acid and titrated against 10 ml of mixed Fehlings solution (5 ml Fehling A + 5 ml Fehling solution B) using methylene blue as indicator. Results were expressed as per cent total sugar.

3.7.2.3.3. Non reducing sugars

The amount of non reducing sugar was calculated by subtracting reducing sugars from total sugar and multiplying the difference by factor 0.95 as suggested by AOAC (1980) and expressed as per cent non-reducing sugar.

3.6.2.3.4. Moisture (per cent)

The moisture content wass determined as per AOAC (1984). Weighed amount (5g) of powder was trasferred to a cleaned, dried (at 105 Degree celcius for 20 minutes) and weighed aluminium dish. The contents were dried in oven at 102 degree celcius for 12 hours and cooled in a desiccator. The loss in weight was taken as moisture content and expressed as percentage of moisture by the following formula:

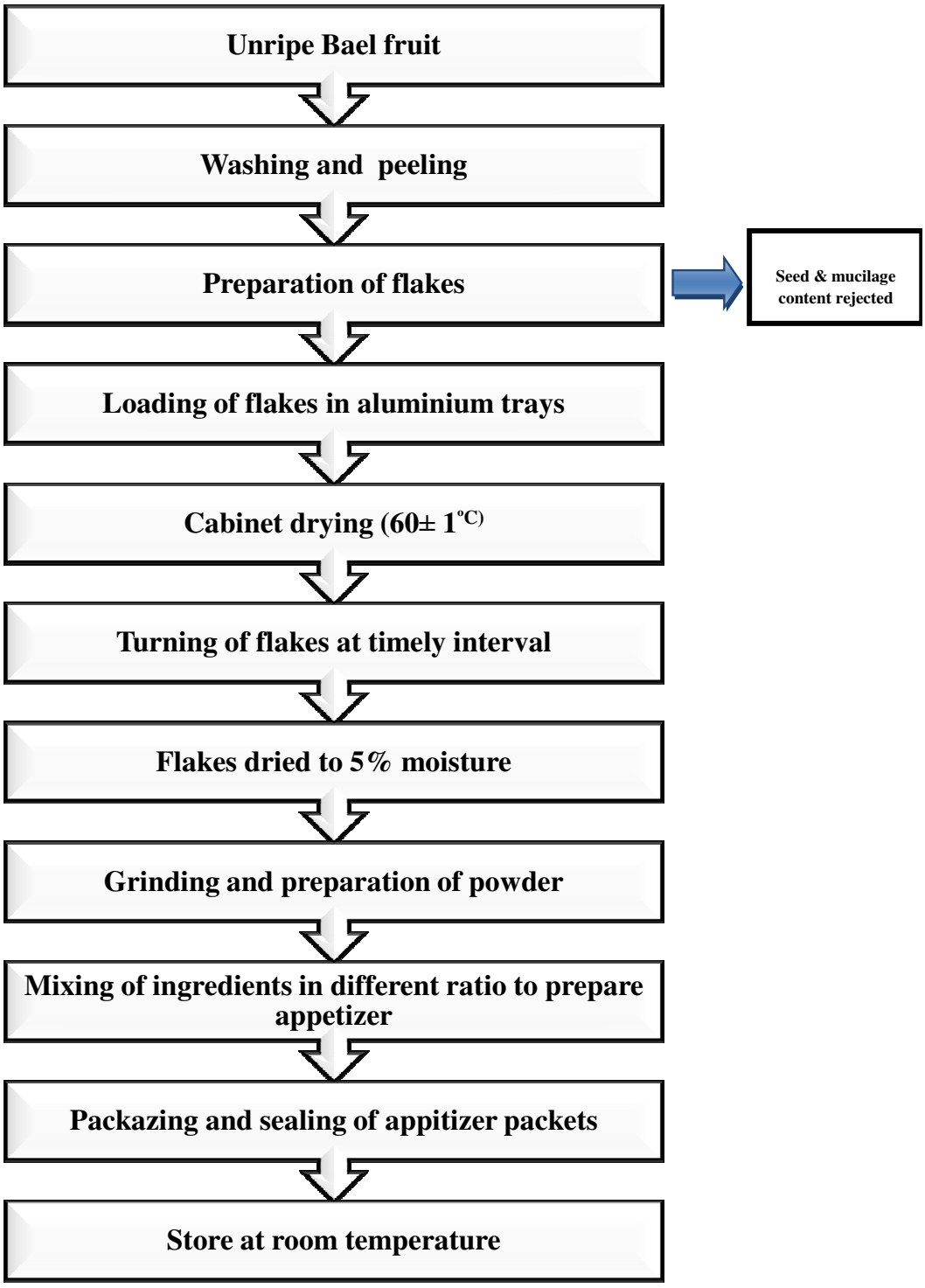


Fig. 3a. Flow diagram for the preparation of Bael fruit powder & preparation of Appetizer

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

3.6.2.3.5. Titratable acidity

The titrable acidity was determined by titrating the 10 ml aliquot against 0.1N sodium hydroxide solution using phenolphthalein as indicator by method as suggested by AOAC (1980). The result was expressed as per cent citric acid.

3.6.2.3.6. Non enzymatic browning

Non-enzymatic browning estimation to a known quantity of Bael powder (10 g), 10 ml of water and 30 ml of absolute alcohol were added and thoroughly mixed. After keeping it overnight. the content was filtered and measured for the colour at 440 nm in spectrophotometer and 60 per cent alcohol was used as blank (Ranganna, 1995).

3.6.2.4. Sensory analysis of appetizer

Samples of appetizers were presented to a panel of 8 judges. For evaluating the appetizer, nine point hedonic scale was used (Appendix VII). The panelists were briefed about method of scoring before serving the samples. The samples were served at room temperature. The averages were calculated from all the characteristics and total points were tabulated.

3.7.2.5. Storage studies

The packets of appetizers were prepared and stored at room temperature. After four months, storage studies were performed for moisture and non enzymatic browning by the methods as described earlier.

3.6.3. Statistical analysis:

Biochemical and quality analysis data were subjected to statistical analysis by using CRD and FCRD. The mean difference was tested by 'F'test at 5 percent level of significance (LOS). Critical difference (CD) at 5 per cent level of probability was used for comparison among treatments. The results were presented by way of tables and figures. Analysis of quantitative data (biochemical and quality analysis) were done in statistical tool OPSTAT**,** Statistical Software Package for Agricultural Research, CCSHAU, Hissar.

3.6.4. Analysis of molecular data

Molecular data was interpreted using NTSYS-pc version 2.11s (Rohlf, 2000), for generating UPGMA dendrogram. The following values were calculated which have been described under respective subheads.

3.6.4.1. Polymorphic Information Content

Polymorphic information content (PIC) that provides an estimate of the discriminatory power of a locus or loci, by taking into account, not only the number of alleles that expressed, but also relative frequencies of those alleles, values were calculated for each SSR primers according to the formula given by Smith *et al*. (1997): PIC = 1- $\Sigma P i^2$, Where $P i^2$ is the frequency of the *ith* allele.

3.6.4.2. Percentage polymorphism

Percentage polymorphism is calculated for each primer separately which describes its discriminatory power. It is calculated by dividing the number of polymorphic amplicons with the total number of amplicons amplified by that primer *i.e.,*

$$\text{Percentage polymorphism} = \frac{\text{Number of polymorphic loci/amplcons}}{\text{Total number of loci/amplicons}}$$

3.6.4.3. Heterozygosity

Heterozygosity is the state of possessing different alleles at a given locus in regard to a given character. It is a measure of heterozygotes or genetic variation in a population. The population heterozygosity calculated as method given by (Nei, 1978).

3.6.4.4. Similarity coefficient and construction of dendrogram

The amplification product was scored separately in the form of binary matrix for each primer on the basis for the presence (1) or absence (0) of bands of various molecular weight sizes DNA fragments. Data were analyzed to obtain Jaccard‟s similarity coefficients among the isolates by using NTSYS-pc version 2.11s (Rohlf, 2000). The SIMQUAL program was used to calculate the Jaccard‟s similarity coefficients.

$$\text{Jaccard‟s similarity coefficients} = N\text{AB}/(N\text{AB} + N\text{A} + N\text{B})$$

Where, *N*AB is the number of bands shared by samples, *N*A represents amplified fragments in sample A, and *N*B represents amplified fragments in sample B. Similarity

matrices based on these indices were obtained. All the gels were scored twice manually and independently. All unique bands was also scored and included in the analysis. The similarity coefficients were then used to construct a dendrogram manually by UPGMA (Unweighted pair-group method with arithmetical averages) was calculated by formulae:

$$\text{Coefficient of similarity} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}}$$

Based on the similarity coefficients, separate dendrogram was prepared for SSRs markers. Cluster analysis was performed and dendrogram was plotted based on SSRs markers data among 24 genotypes of Bael, following unweighted pair group method with arithmetical averages.

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

The results of the present investigation entitled "Biochemical and molecular characterization of diverse genotype of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties " have been presented in this chapter under the appropriate heads and sub-heads. The observations recorded during the course of experiment are statistically analysed and presented in the form of tables and figures. The results have been discussed and supported by available literature under the following headings.

4.1. Effect of genotypes on biochemical parameters

4.1.1. Non enzymatic antioxidants

The non enzymatic antioxidants also refer as dietary antioxidants have potential beneficial effects in protecting against diseases which was well established phenomenon. Today the emerging concept is that nutrition may play a significant role in helping to defend against the oxidative stress and the damage induced by free radicals. The certain dietary components and nutrients with antioxidant properties are imperative for the protection against oxidative stress injury to body. In normal conditions, our cell can capable to prevent free radical induced diseases by generation its own endogenous antioxidants or by take them from food. Therefore, non enzymatic antioxidants like ascorbic acid, falvonoids, carotenoids, phenols and other through our diet play an important role in helping endogenous antioxidants for the neutralization of excess free radicals. Bael is well known for its therapeutic and nutritional potential; hence different non enzymatic antioxidants are studied in diverse genotype of Bael.

4.1.1.1. Ascorbic acid

The data regarding ascorbic acid in diverse genotypes of Bael is presented in Table 4.1 and graphically illustrated in Fig.4a. A significant variation was observed among the genotypes in respect of ascorbic acid content. The ascorbic acid content ranged from 9.11 to 20.30mg/100g among the genotypes. The maximum ascorbic acid content was observed from Pant Bael-3 (20.30mg/100g), which was found at par with Pant Bael-1 (18.60mg/100g). However, the minimum ascorbic acid content was obtained from Haldi Nurmohamd (9.11mg/100g).

Table 4.1 : Content of ascorbic acid (mg/100g) in diverse genotypes of Bael

Treatments	Ascorbic acid (mg/100g)
T_1 : (Pant Bael-1)	18.60
T_2 : (Pant Bael-2)	15.50
T_3 : (Pant Bael-3)	20.30
T_4 : (Pant Bael-4)	15.50
T_5 : (Pant Shivani)	13.90
T_6 : (Pant Urvashi)	10.20
T_7 : (Haldi Nurmohamd)	9.11
T_8 : (Patharchatta-1)	12.01
T_9 : (Faizabad No.9)	13.44
T_{10} : (Faizabad Local)	12.90
T_{11} : (Pant Aparna)	14.45
T_{12} : (Pant Bael-10)	11.01
T_{13} : (Pant Sujata)	16.89
T_{14} : (Pant Bael-13)	10.85
T_{15} : (Pant Bael-14)	13.56
T_{16} : (Pant Bael- 15)	14.31
T_{17} : (Pant Bael -16)	12.34
T_{18} : (Gonda No.2)	12.78
S.Em. ±	0.55
C.D. at 5%	2.03

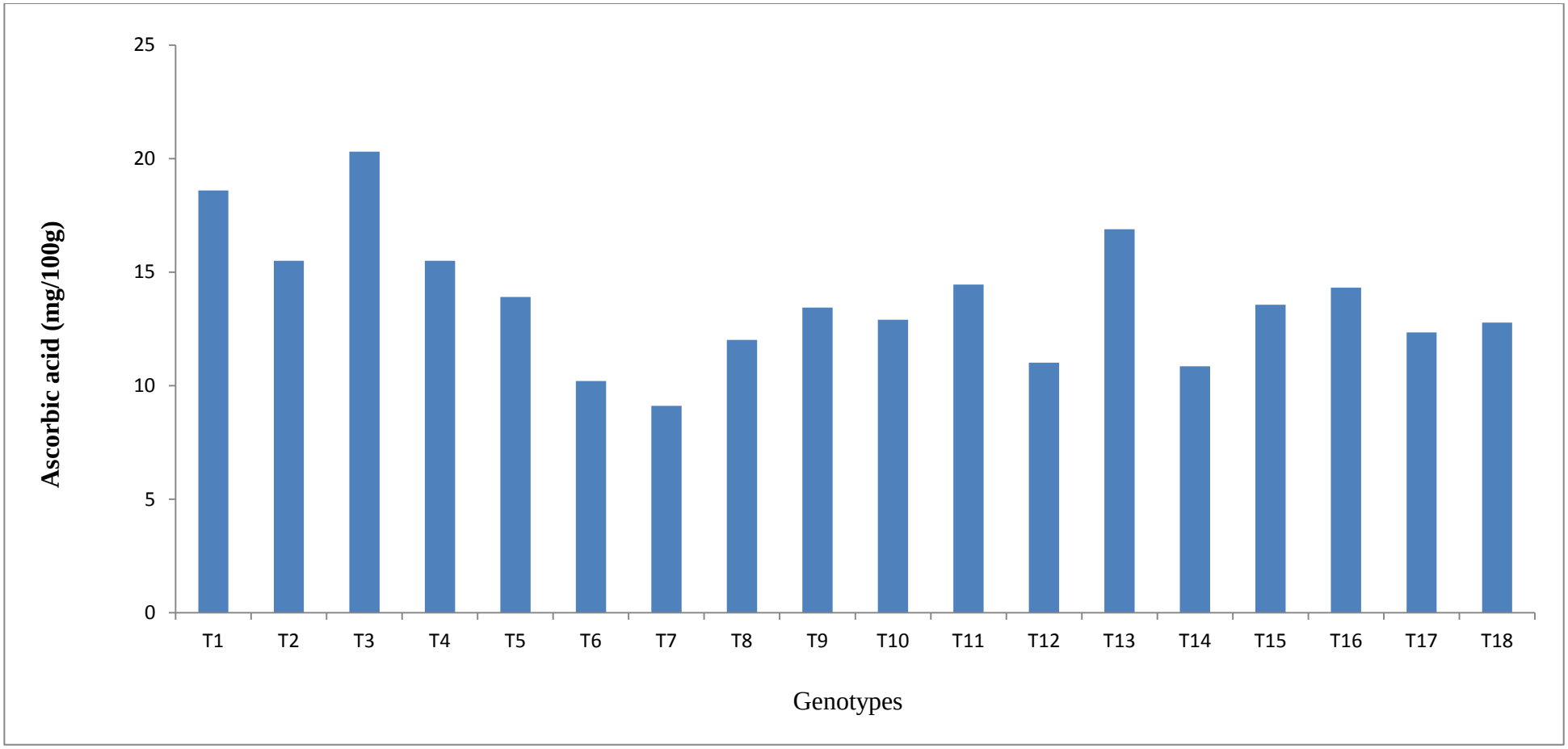


Fig.4a. Ascorbic acid content of diverse genotypes of Bael

The similar effect of genotype on ascorbic acid content of Bael is reported by Teaotia *et al.* (1963); Jauhari *et al.* (1969); Roy and Singh, (1978); Srivastava and Singh, (2004); Saroj *et al.* (2008) and Pavani *et al.* (2018). This variation in ascorbic acid content among the Bael genotypes is due to the variation in genetic composition and its adaptation to the agroclimatic conditions. The values of ascorbic acid in Bael signify potential use of the fruit as a good natural source of ascorbic acid. The recommended daily intake (RDI) of ascorbic acid is about 30 mg/day for adults and 17 mg/day for children. Therefore, its fruits could be considered as good sources of ascorbic acid for purposes of human nutrition.

4.1.1.2. Marmelosin

The data presented in Table 4.2 and graphically shown in Fig.4b clearly depict that marmelosin content of Bael fruits were significantly varied among its different genotypes. The value of marmelosin ranged from 0.08 to 1.29mg/g. The maximum value of marmelosin is found in Pant Bael -1(1.29mg/g), which was found at par with Pant Bael -15/g (1.25mg) and Pant Bael- 13(1.13mg/g). Whereas, the minimum value of marmelosin is obtained from Haldi Nurmohamd-2 (0.08mg/g). Marmelosin also called as imperatorin is a major chemical constituent of the Bael fruit and has been proved to have an anticancer, antibacterial and anti-inflammatory activity.

Shailajan *et al.,* (2012) found that, mamelosin concentration in Bael fruit pulp is 1.251+0.0069 mg/g. Shinde and Laddha, (2014) reported that, marmelosin content in Bael fruit is 5-6.5%. The effect of genotype on marmelosin content of Bael fruit is not reported in past. Marmelosin is a naturally occurring phytochemical with high therapeutic potential founds in Bael. Marmelosin is natural coumarins that are heterocyclic molecules that have been associated with beneficial effects on human health, due to their antioxidant activities. Beside this, it is antidiabetic agent (Prajapat *et al.* 2012), it possess anthelminticactivity (Venugopal *et al.* 2013), hepatoprotective and antidiarrhoel activities (Shailajan *et al.* 2012)

4.1.1.3. Total phenol content

The perusal of data in Table 4.3 and Fig. 4c indicates that there was a significant effect of genotypes on total phenol content of Bael fruits. The value of total phenol content ranged from 51.50 to 105.00 mg GAE /g. The maximum total phenol content in noted from Pant Sujata (105.00mg GAE /g), which was found significantly superior other

Table 4.2: Content of marmelosin (mg/g) in diverse genotypes of Bael

Treatments	Marmelosin (mg/g)
T_1 : (Pant Bael-1)	1.29
T_2 : (Pant Bael-2)	0.90
T_3 : (Pant Bael-3)	0.60
T_4 : (Pant Bael-4)	0.62
T_5 : (Pant Shivani)	0.61
T_6 : (Pant Urvashi)	0.28
T_7 : (Haldi Nurmohamd)	0.08
T_8 : (Patharchatta-1)	0.23
T_9 : (Faizabad No.9)	0.58
T_{10} : (Faizabad Local)	0.58
T_{11} : (Pant Aparna)	0.30
T_{12} : (Pant Bael-10)	0.44
T_{13} : (Pant Sujata)	0.67
T_{14} : (Pant Bael-13)	1.13
T_{15} : (Pant Bael-14)	0.50
T_{16} : (Pant Bael- 15)	1.25
T_{17} : (Pant Bael -16)	0.59
T_{18} : (Gonda No.2)	0.65
S.Em. ±	0.06
C.D. at 5%	0.21

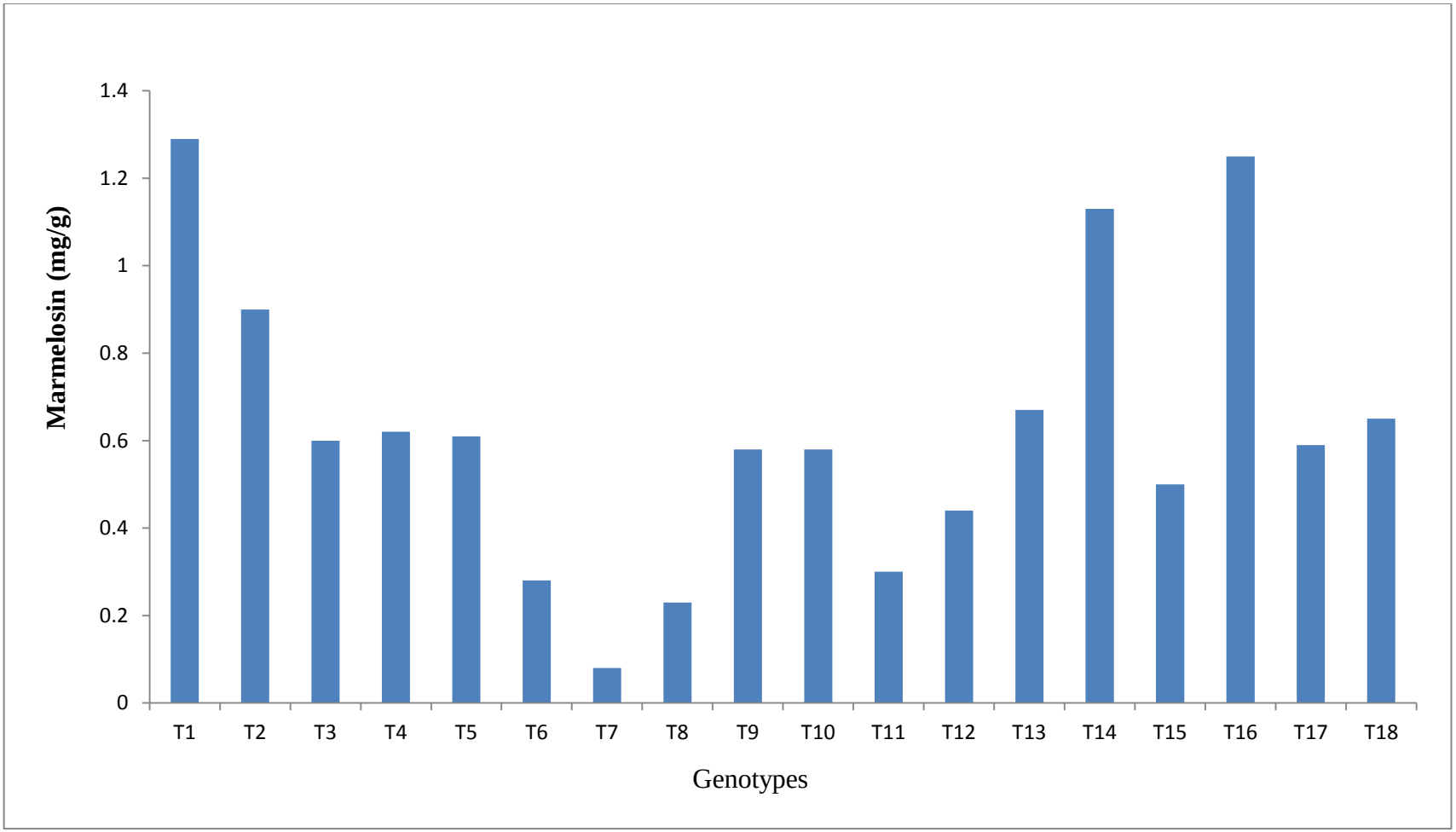


Fig.4b. Marmelosin content of diverse genotypes of Bael

Table 4.3: Content of total phenol content (mg GAE/g) in diverse genotypes of Bael

Treatments	Total phenol content (mg GAE/g)
T_1 : (Pant Bael-1)	77.60
T_2 : (Pant Bael-2)	90.40
T_3 : (Pant Bael-3)	95.20
T_4 : (Pant Bael-4)	98.80
T_5 : (Pant Shivani)	84.50
T_6 : (Pant Urvashi)	77.00
T_7 : (Haldi Nurmohamd)	76.65
T_8 : (Patharchatta-1)	62.45
T_9 : (Faizabad No.9)	78.80
T_{10} : (Faizabad Local)	77.50
T_{11} : (Pant Aparna)	101.50
T_{12} : (Pant Bael-10)	83.75
T_{13} : (Pant Sujata)	105.00
T_{14} : (Pant Bael-13)	78.35
T_{15} : (Pant Bael-14)	97.70
T_{16} : (Pant Bael- 15)	82.05
T_{17} : (Pant Bael -16)	51.50
T_{18} : (Gonda No.2)	89.45
S.Em. ±	0.58
C.D. at 5%	2.13

genotype in respect to phenol content. However, the minimum total phenol content is obtained from the Pant Bael-16 (51.50 GAE mg/g).

Jain *et al.* (2011) noted that phenol content of Bael fruit was 95.33 mg GAE/g. Charoensiddhi and Anprung (2008), also found that phenolic contents of Thai Bael fruit was 87.34 mg GAE/ g. Kharub *et al.* (2014) revealed that the different varieties of Bael exhibited considerable physico-chemical attributes of Bael fruits. The total phenols content of different Bael varieties is ranges from 2.34-2.75%.

Similar effect of genotype on phenol content was reported in other fruit crops by Cevallos-Casals *et al.* (2006) in plum and peach genotypes, Khanizadeh *et al.* (2008) and Matthes and Schmitz-Eiberger, (2009) in apple, Deshmukh *et al.* (2009) in banana, Panico *et al.*(2009) in strawberry,Sochor *et al.* (2010) and Caliskan*et al.* (2012) in apricot, Lu *et al.*(2014) in pineapple andKoley *et al.* (2016) in ber.

4.1.1.4. Pectin content

The data on pectin content reveals that there was significant difference on pectin content among the diverse genotype of Bael (Table 4.4 and Fig.4d). The range of pectin content is from 3.96-19.12%. The maximum pectin content was obtained from Haldi Nurmohamd (19.12%) which was found at par with Pant Bael- 3 (18.96%) and Pant Bael-16(17.04%). The minimum pectin content was obtained from Pant Bael -2 (3.96%). Singh *et al.* (2012) found that pectin content in Bael pulp was 8.8 g/100g. Anup *et al.* (2017) studied the pectin transitions during growth and development of CISH B-1 and CISH B-2 and observed significance differences. Besides the antioxidant activity of pectin, it is used in a number of foods as a gelling agent, emulsifier, stabilizer, texturizer and thickener.

Bael fruits are rich source of pectin. An study on Bael fruit pectin revealed that, absence of hemagglutinating activity and antinutritional factors together with the activity to confer better emulsion capacity, stability and antimicrobial activity gives Bael fruit pectin a clear edge over commercial citrus pectin for exploitation as an additive in food and pharmaceuticals (Jindal *et al.* 2013).The similar effect of genotypes in pectin content of fruit was also reported by Lopez *et al.* (1958) in apple, Deshmukh *et al.* (2009) in banana, Mathur *et al.* (2011) in apple and Singh *et al.* (2016) in guava.

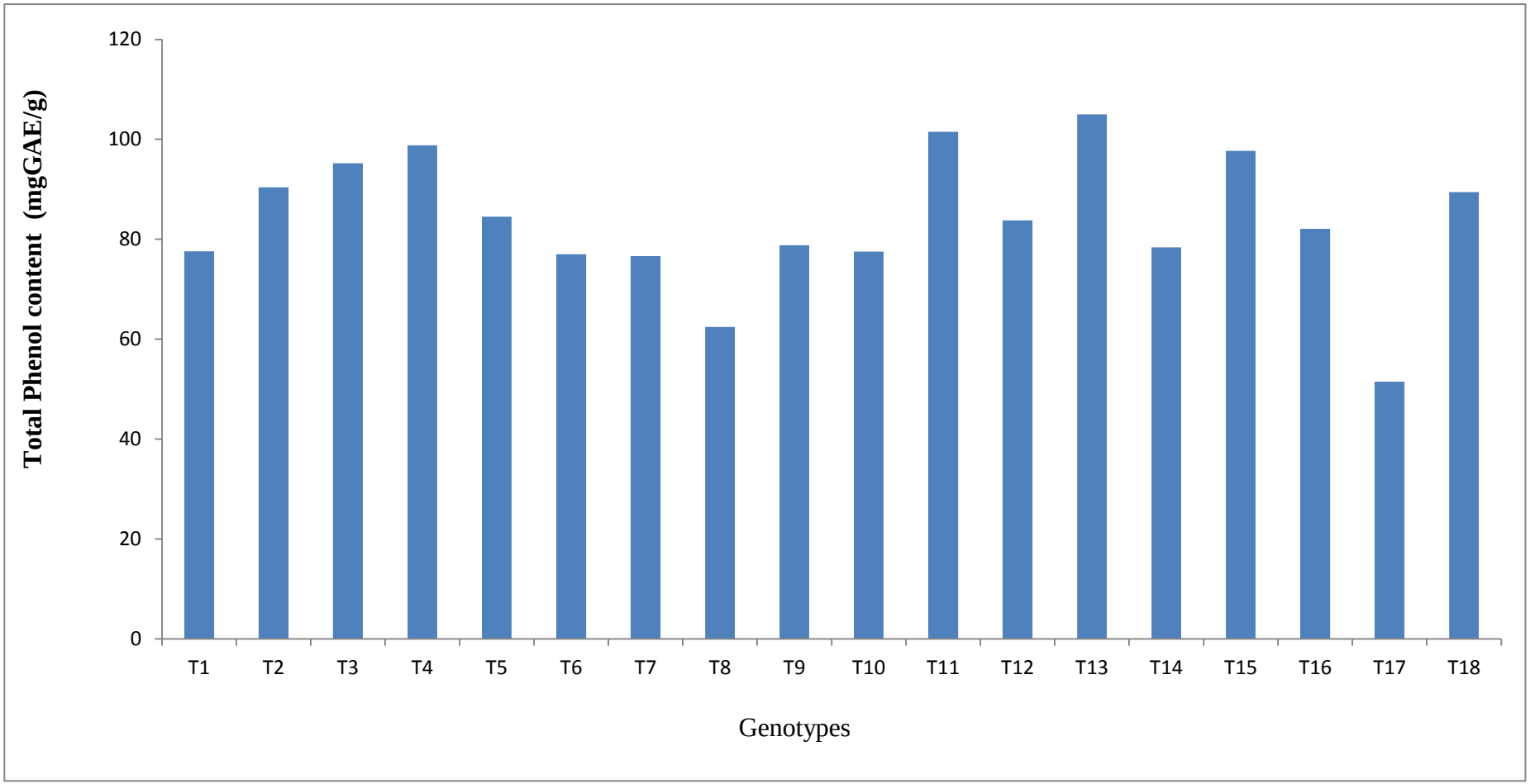


Fig.4c. Total Phenol content of diverse genotypes of Bael

Table 4.4: Content of pectin content (%) in diverse genotypes of Bael

Treatments	Pectin content (%)
T_1 : (Pant Bael-1)	5.04 (2.24)
T_2 : (Pant Bael-2)	3.96 (1.99)
T_3 : (Pant Bael-3)	18.96 (4.35)
T_4 : (Pant Bael-4)	4.44 (2.11)
T_5 : (Pant Shivani)	5.52 (2.35)
T_6: (Pant Urvashi)	6.56 (2.56)
T_7 : (Haldi Nurmohamd)	19.12 (4.37)
T_8 : (Patharchatta-1)	13.44 (3.67)
T_9 : (Faizabad No.9)	13.92 (3.73)
T_{10} : (Faizabad Local)	13.44 (3.67)
T_{11} : (Pant Aparna)	9.72 (3.12)
T_{12} : (Pant Bael-10)	9.54 (3.09)
T_{13} : (Pant Sujata)	9.68 (3.11)
T_{14} : (Pant Bael-13)	10.32 (3.21)
T_{15} : (Pant Bael-14)	12.24 (3.50)
T_{16} : (Pant Bael- 15)	10.92 (3.30)
T_{17} : (Pant Bael -16)	17.04 (4.13)
T_{18} : (Gonda No.2)	10.09 (3.18)
S.Em. ±	(0.176)
C.D. at 5%	(0.506)

(Figures shown in parenthesis are square root transformed value)

4.1.1.5. Riboflavin

The data on Table 4.5 and Fig.4e show that, significant effect of genotypes on riboflavin in Bael fruit. The value of riboflavin ranged from 0.19 to 1.21 mg/100g. The highest riboflavin was found in Pant Sujata (1.21mg/100g). It was also found at par with Pant Aparna and Pant Shivani in which riboflavin were 1.05 mg/100g and 1.01mg/100g, respectively. The lowest riboflavin was noted in Haldi Nurmohamd (0.19mg/100g). Similar, effect of genotype on riboflavin content of fruit revealed by Bari *et al.* (2006) in papaya, Englberger *et al.* (2010) in banana and Yada *et al.* (2013) in almond. The values of ribofalvin in Bael signify potential use of the fruit as a good natural source of riboflavin. The recommended daily intake (RDI) of riboflavin is 1.3 milligrams daily for men and 1.1 mg for women. Therefore, Bael fruits are considered as good sources of riboflavin for purposes of human nutrition.

4.1.1.6. Total Carotenoids

Data on total carotenoids presented in Table 4.6 and Fig.4f revealed that, significant effect of genotypes on total carotenoids of Bael fruits. The value of total carotenoids were ranged from 0.96mg to 5.11mg/100g. The maximum total carotenoids were noted from Pant Bael -1 (5.11mg/100g) which was found at par with Pant Bael-2 (3.86mg/100g). Whereas, the minimum total carotenoids was noted in Pant Bael-10 (0.96mg/100g).

Similar findings which shows the effect of genotypes on total carotenoids also reported in different fruits byUdumalagala Gamage *et al.* (2003) in papaya, Ruiz *et al.* (2005) in apricot, Englberger *et al.*(2010) in banana, Haque *et al.* (2015) andBora *et al.* (2017) in mango.

4.1.1.7. Total Flavonoids

The perusal of data in Table 4.7 and Fig.4g clearly shows that the, the effect of genotype on total flavonoids was found to be significant. The value of total flavonoids ranged from 32.28 to 75.08 mg catechin/g Dw among the different genotypes. The highest total flavonoids was observed in Pant Aparna (75.08 mg catechin/g Dw) which was found significantly superior over other genotypes followed by Pant Sujata and Pant Shivani in which total flavonoids were 63.65 and 52.28 mg catechin/g Dw repectively. However, the lowest total flavonoids was found in Patharchatta -1 (32.28 mg catechin/g Dw).

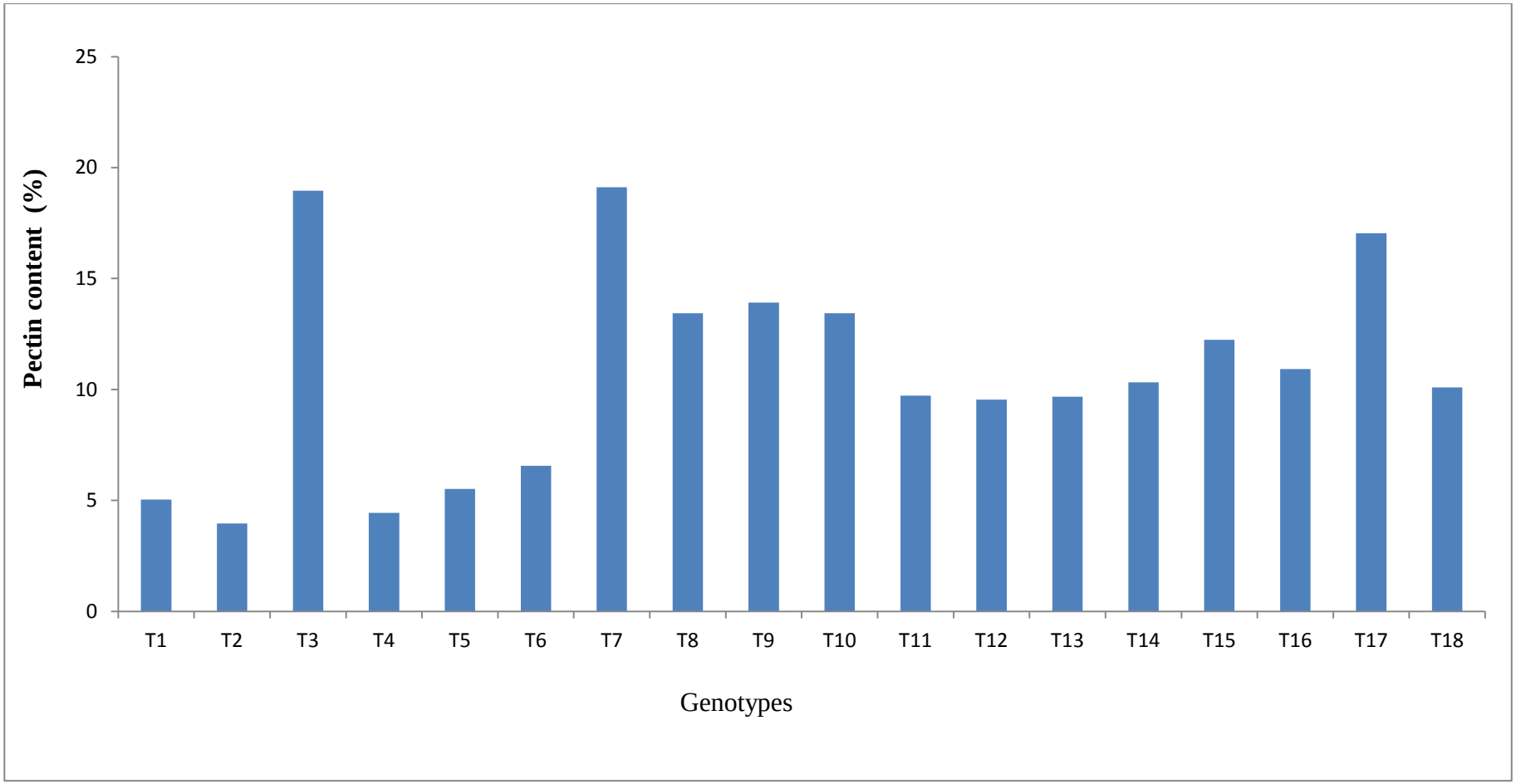


Fig.4d. Pectin content in diverse genotypes of Bael

Table 4.5: Content of riboflavin (mg/100g) in diverse genotypes of Bael

Treatments	Riboflavin (mg/100g)
T_1 : (Pant Bael-1)	0.80
T_2 : (Pant Bael-2)	0.45
T_3 : (Pant Bael-3)	0.30
T_4 : (Pant Bael-4)	0.38
T_5 : (Pant Shivani)	1.01
T_6 : (Pant Urvashi)	0.93
T_7 : (Haldi Nurmohamd)	0.19
T_8 : (Patharchatta-1)	0.34
T_9 : (Faizabad No.9)	0.65
T_{10} : (Faizabad Local)	0.67
T_{11} : (Pant Aparna)	1.05
T_{12} : (Pant Bael-10)	0.57
T_{13} : (Pant Sujata)	1.21
T_{14} : (Pant Bael-13)	0.59
T_{15} : (Pant Bael-14)	0.55
T_{16} : (Pant Bael- 15)	0.32
T_{17} : (Pant Bael -16)	0.44
T_{18} : (Gonda No.2)	0.73
S.Em. ±	0.075
C.D. at 5%	0.276

Table 4.6: Content of total carotenoid (mg/100g)in diverse genotypes of Bael

Treatments	Total carotenoids (mg/100g)
T_1 : (Pant Bael-1)	5.11
T_2 : (Pant Bael-2)	3.86
T_3 : (Pant Bael-3)	2.50
T_4 : (Pant Bael-4)	1.25
T_5 : (Pant Shivani)	2.12
T_6: (Pant Urvashi)	1.54
T_7 : (Haldi Nurmohamd)	1.25
T_8 : (Patharchatta-1)	1.25
T_9 : (Faizabad No.9)	1.74
T_{10} : (Faizabad Local)	2.81
T_{11} : (Pant Aparna)	1.60
T_{12} : (Pant Bael-10)	0.96
T_{13} : (Pant Sujata)	2.31
T_{14} : (Pant Bael-13)	1.63
T_{15} : (Pant Bael-14)	1.54
T_{16} : (Pant Bael- 15)	1.74
T_{17} : (Pant Bael -16)	1.06
T_{18} : (Gonda No.2)	1.16
S.Em. ±	0.34
C.D. at 5%	1.25

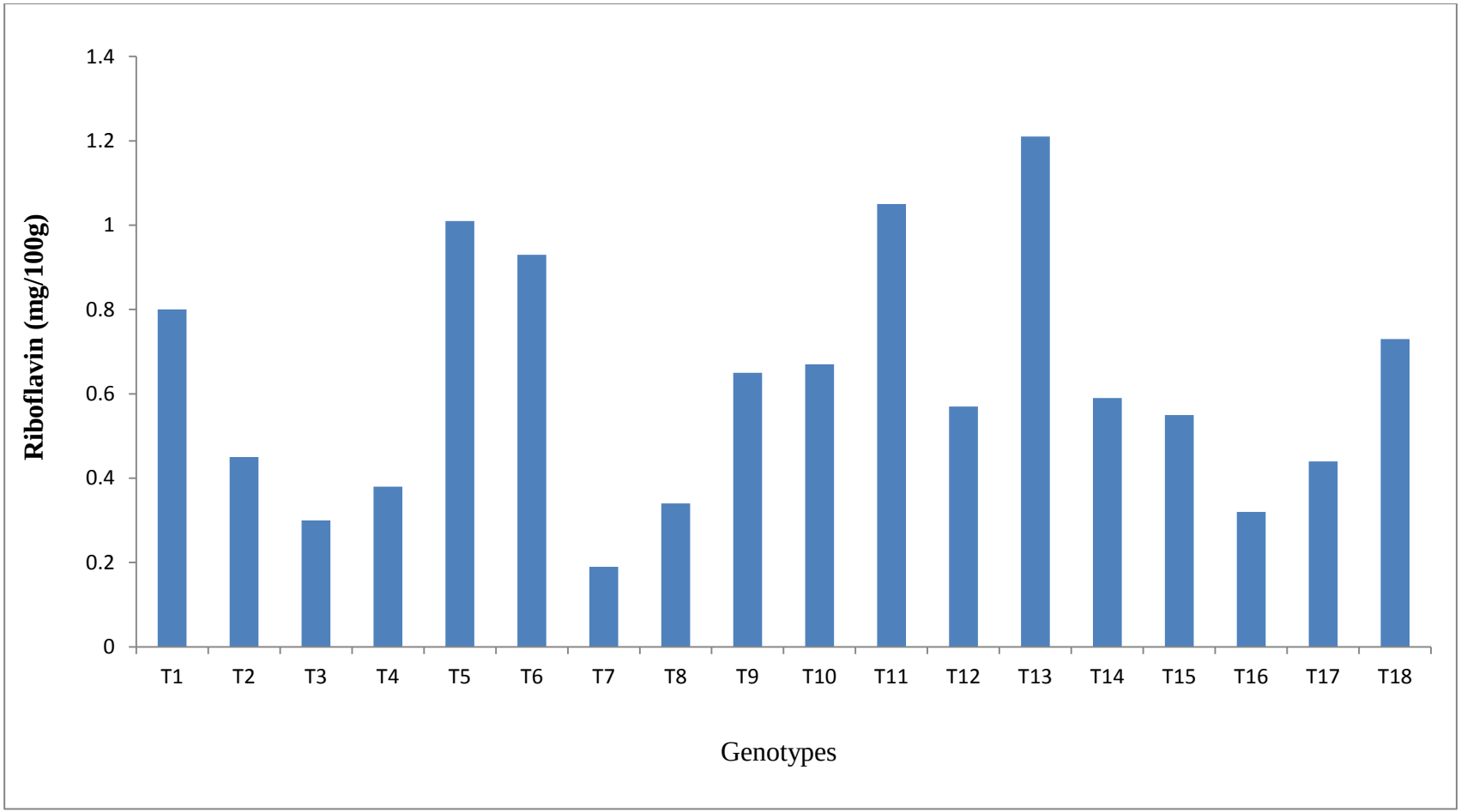


Fig.4e. Riboflavin in diverse genotypes of Bael

Table 4.7: Content of Total flavonoid (mg catechin/g DW)in diverse genotypes of Bael

Treatments	Total Falvonoid (mg catechin/g DW)
T_1 : (Pant Bael-1)	42.88
T_2 : (Pant Bael-2)	41.00
T_3 : (Pant Bael-3)	44.70
T_4 : (Pant Bael-4)	44.55
T_5 : (Pant Shivani)	52.28
T_6 : (Pant Urvashi)	43.75
T_7 : (Haldi Nurmohamd)	36.30
T_8 : (Patharchatta-1)	32.28
T_9 : (Faizabad No.9)	42.45
T_{10} : (Faizabad Local)	45.05
T_{11} : (Pant Aparna)	75.08
T_{12} : (Pant Bael-10)	45.85
T_{13} : (Pant Sujata)	63.65
T_{14} : (Pant Bael-13)	40.03
T_{15} : (Pant Bael-14)	41.88
T_{16} : (Pant Bael- 15)	42.88
T_{17} : (Pant Bael -16)	45.98
T_{18} : (Gonda No.2)	44.90
S.Em. ±	0.63
C.D. at 5%	2.33

Germana*et al.* (2004) in citrus, Javdani *et al.* (2013) in apple, Lu *et al.* (2014) inpineapple, Faramarz *et al.* (2015) in oleaster and Hua *et al.* (2018) in mandarin also reported the variation among genotype regarding total flavonoids.

4.1.2. Enzymatic antioxidants

The superoxide dismutase (SOD), peroxidase (POD) and catalase are the key enzymatic antioxidants by which the free radicals that are produced during oxidative stresses are removed. These oxidative stresses lead to many serious human diseases. Superoxide dismutases are catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Catalase and peroxidise enzyme catalyze the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is harmful by-product of many normal metabolic processes. The enzymatic antioxidants superoxidase dismutase (SOD), peroxidase (POD) and catalase (CAT) activity studied in different genotype of Bael and significant variation was observed. The data pertaining the enzymatic antioxidants activity are presented in Table 4.8.

4.1.2.1. Superoxidase dismutase (SOD)

The data pertaining the superoxidase dismutase activity as influenced by the diverse genotype of Bael presented in Table 4.8. The maximum superoxidase dismutase enzyme activity was noted from Pant Shivani (31.06U/g Fw). However, the lowest superoxidase dismutase enzyme activity was noted in Faizabad Local (11.50 U/g Fw).

4.1.2.2. Catalase (CAT)

The perusal of data in Table 4.8 indicates that there was a significant difference among genotype in respect to catalase activity. The highest catalase enzyme activity was reported from Pant Shivani (5.47 ΔO.D./ min/g Fw) which was found at par with Pant Bael-10 (5.20 ΔO.D./ min/g Fw) and Pant Bael -4 (4.72 ΔO.D./ min/g Fw). However, the lowest catalase enzyme activity was noted in Patharchatta -1 (2.21 ΔO.D./ min/g Fw).

4.1.2.3. Peroxidase (POD)

The data on peroxidise activity reveals that there was significant difference among diverse genotype of Bael (Table 4.8). The highest peroxidase enzyme activity was observed from Pant Bael -1(6.55 ΔO.D. /min/g Fw). Whereas, the lowest peroxidise enzyme activity was foundin Pant Bael-2(0.44ΔO.D. /min/g Fw).

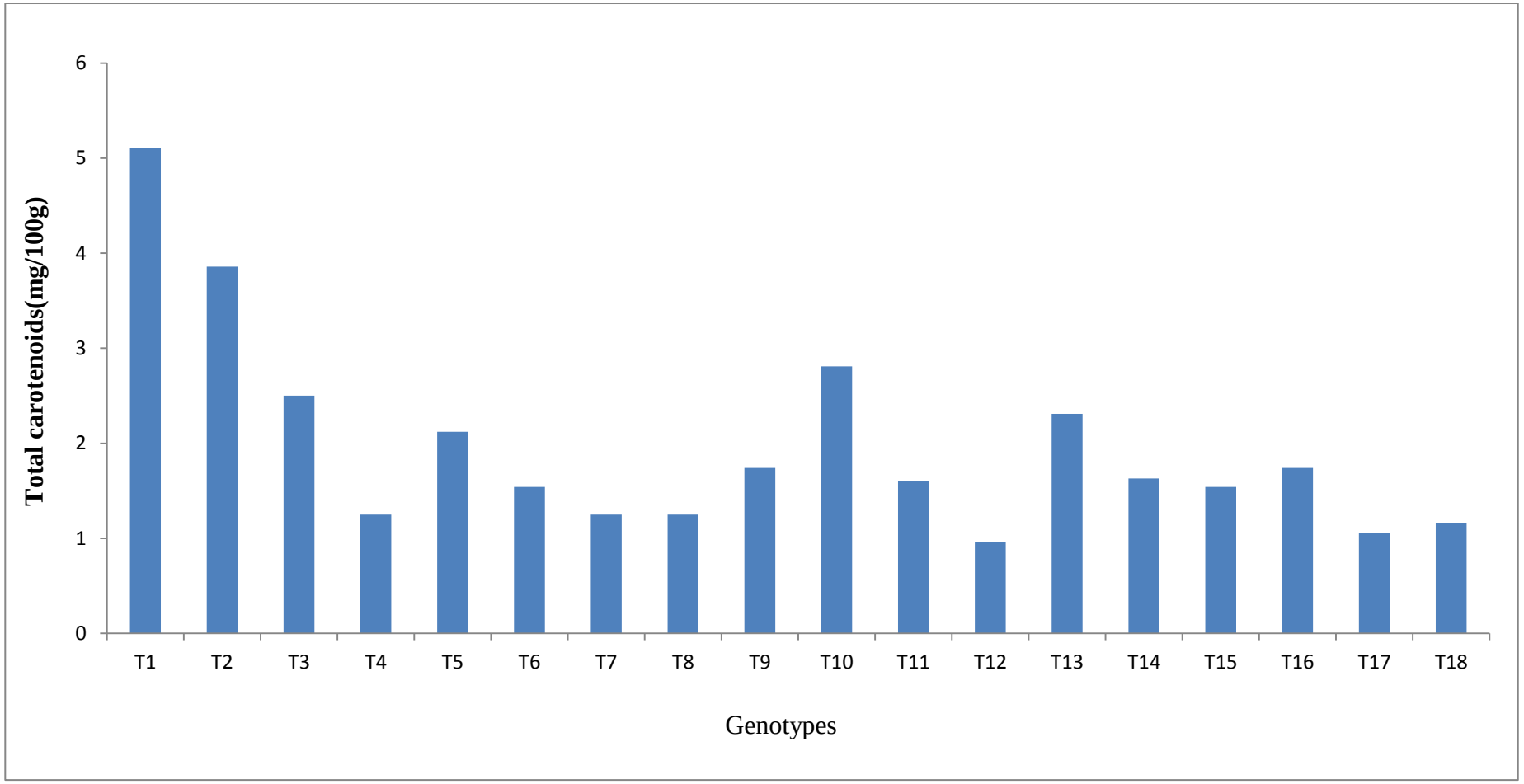


Fig. 4f. Total carotenoids in diverse genotypes of Bael

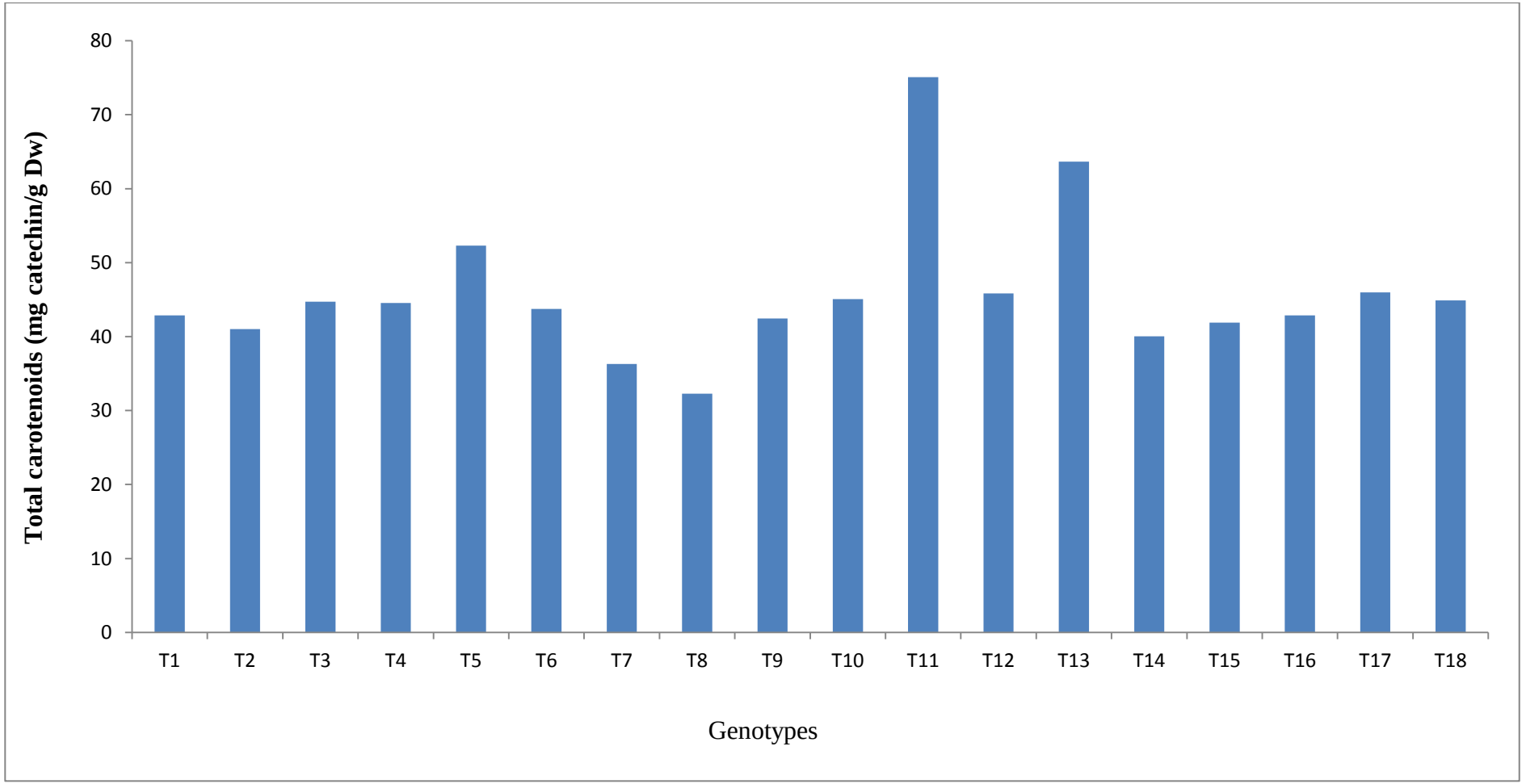


Fig. 4g. Total flavonoids in diverse genotypes of Bael

Table 4.8: Enzymatic antioxidant activity in diverse genotype of Bael

Treatments	SOD (U/g Fw)	CAT(ΔO.D./min/g Fw)	POD((ΔO.D /min/g Fw)
T_1 : (Pant Bael-1)	13.91	4.08	6.55
T_2 : (Pant Bael-2)	27.89	3.58	0.44
T_3 : (Pant Bael-3)	28.24	2.77	1.03
T_4 : (Pant Bael-4)	22.04	4.72	0.66
T_5 : (Pant Shivani)	31.06	5.47	4.35
T_6 : (Pant Urvashi)	27.19	4.46	5.17
T_7 : (Haldi Nurmohamd)	26.49	3.54	5.83
T_8 : (Patharchatta-1)	23.70	2.21	0.96
T_9 : (Faizabad No.9)	27.41	4.11	1.55
T_{10} : (Faizabad Local)	11.58	3.61	4.72
T_{11} : (Pant Aparna)	26.98	3.61	3.69
T_{12} : (Pant Bael-10)	24.19	5.20	4.50
T_{13} : (Pant Sujata)	28.80	3.20	5.09
T_{14} : (Pant Bael-13)	16.19	4.55	1.92
T_{15} : (Pant Bael-14)	18.93	4.57	4.72
T_{16} : (Pant Bael- 15)	18.38	4.40	5.39
T_{17} : (Pant Bael -16)	25.19	2.70	1.85
T_{18} : (Gonda No.2)	27.51	3.60	4.06
S.Em. ±	**0.005**	0.373	0.133
C.D. at 5%	0.017	1.081	0.383

The similar effect of genotype on different enzymatic antioxidants also reported by Lata *et al.* (2005) in apple, Vanini*et al.* (2010) in avocado,De souza *et al.*(2014) in acerola, and Lopes *et al.* (2015) in cashew apple.

4.1.3. DPPH radical scavenging activity (%)

The data on DPPH radical scavenging activity presented in Table 4.9 and Fig.4h show that DPPH radical scavenging activity was significantly varied among the various genotypes. The maximum DPPH radical scavenging activity reported from Pant Shivani (81.10%) followed by Haldi Nurmohamd (80.84%) and Faizabad Local (80.62%). Whereas, the minimum DPPH radical scavenging activity observed in Pant Bael-14 (40.32%).

Similar finding which shows the effect of genotype on DPPH radical scavenging activity (%) also reported by Bopitiya and Madhujith, (2012) in pomegranate, Deshmukh *et al.*(2009) in banana, Dantas *et. al*. (2013) in strawberry guava, Lu *et al.* (2014) in pineapple, Faramarz *et al.* (2015) in oleaster, Pal *et al.* (2015) in kiwifruit and Hua *et al*. (2018) in citrus.

4.1.4. Other biochemical parameters

4.1.4.1.Total soluble solid (TSS)

Data presented in Table 4.10 and graphically illustrated in Fig.4i.indicated that significant effect of genotype on Total soluble solid (TSS) content on Bael fruits.The value of TSS ranged from 22 to 40 ^{o}Brix. The maximum TSS was obtained from Pant Bael -15 (40 ^{o}Brix) followed by Pant Bael -2 (39 ^{o}Brix) and Pant Bael -1(36 ^{o}Brix). However, the minimum TSS was obtained from Faizabad No.9 (22 ^{o}Brix).

This might be due to the genetic attribute of particular genotype. Similar finding on effect of Bael genotype on TSS also found by Singh *et al*. (2000). Srivastava and Singh, (2004), Kumar *et al.* (2010), Mitra *et al.*(2010), Singh *et al.* (2014), Jana *et al.* (2014), Rao *et al.* (2014), Uddin *et al.* (2016) and Pavani *et al.* (2018).

4.1.4.2. Phenylalanine ammonia lyase (PAL)

The data on phenylalanine ammonia lyase enzyme acitivity indicates that there was a significant difference in PAL enzyme activity among the different genotype of Bael (Table 4.11 and Fig.4j). The maximum phenylalanine ammonia lyase enzyme acitivity was noted from Pant Aparna (0.314 µmole TCA/mg Fw) followed by Pant Sujata

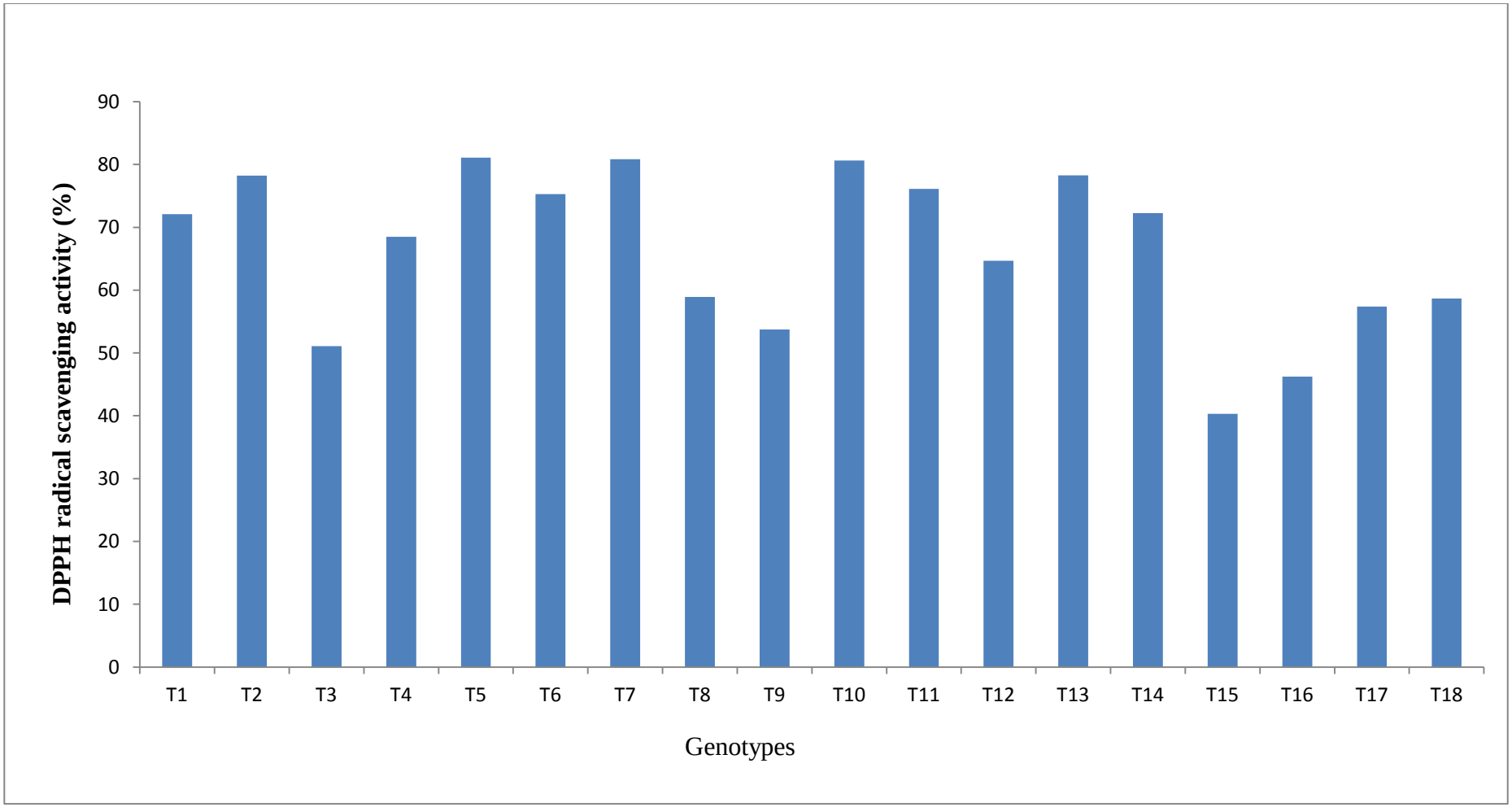


Fig. 4h. DPPH radical scavenging activity of diverse genotype of bael

Table 4.9: DPPH radical scavenging (%) activity of diverse genotype of Bael

Treatments	DPPH radical scavenging (%)
T_1 : (Pant Bael-1)	72.11 (58.12)
T_2 : (Pant Bael-2)	78.21 (62.17)
T_3 : (Pant Bael-3)	51.07 (45.61)
T_4 : (Pant Bael-4)	68.47 (55.84)
T_5 : (Pant Shivani)	81.10 (64.23)
T_6 : (Pant Urvashi)	75.27 (60.18)
T_7 : (Haldi Nurmohamd)	80.84 (64.04)
T_8 : (Patharchatta-1)	58.94 (50.15)
T_9 : (Faizabad No.9)	53.75 (47.15)
T_{10} : (Faizabad Local)	80.62 (63.88)
T_{11} : (Pant Aparna)	76.12 (60.75)
T_{12} : (Pant Bael-10)	64.67 (53.53)
T_{13} : (Pant Sujata)	78.27 (62.21)
T_{14} : (Pant Bael-13)	72.27 (58.22)
T_{15} : (Pant Bael-14)	40.32 (39.42)
T_{16} : (Pant Bael- 15)	46.25 (42.85)
T_{17} : (Pant Bael -16)	57.39 (49.25)
T_{18} : (Gonda No.2)	58.67 (49.99)
S.Em. ±	(0.52)
C.D. at 5%	(1.93)

(Figures shown in parenthesis are Arcsine transformed value)

Table 4.10: Content of TSS (^{o} Brix) in diverse genotypes of Bael

Treatments	TSS(^{o} Brix)
T_1 : (Pant Bael-1)	36
T_2 : (Pant Bael-2)	39
T_3 : (Pant Bael-3)	35
T_4 : (Pant Bael-4)	35
T_5 : (Pant Shivani)	30
T_6: (Pant Urvashi)	29
T_7 : (Haldi Nurmohamd)	30
T_8 : (Patharchatta-1)	25
T_9 : (Faizabad No.9)	22
T_{10} : (Faizabad Local)	30
T_{11} : (Pant Aparna)	28
T_{12} : (Pant Bael-10)	33
T_{13} : (Pant Sujata)	27
T_{14} : (Pant Bael-13)	29
T_{15} : (Pant Bael-14)	30
T_{16} : (Pant Bael- 15)	40
T_{17} : (Pant Bael -16)	30
T_{18} : (Gonda No.2)	29
S.Em. ±	0.314
C.D. at 5%	1.157

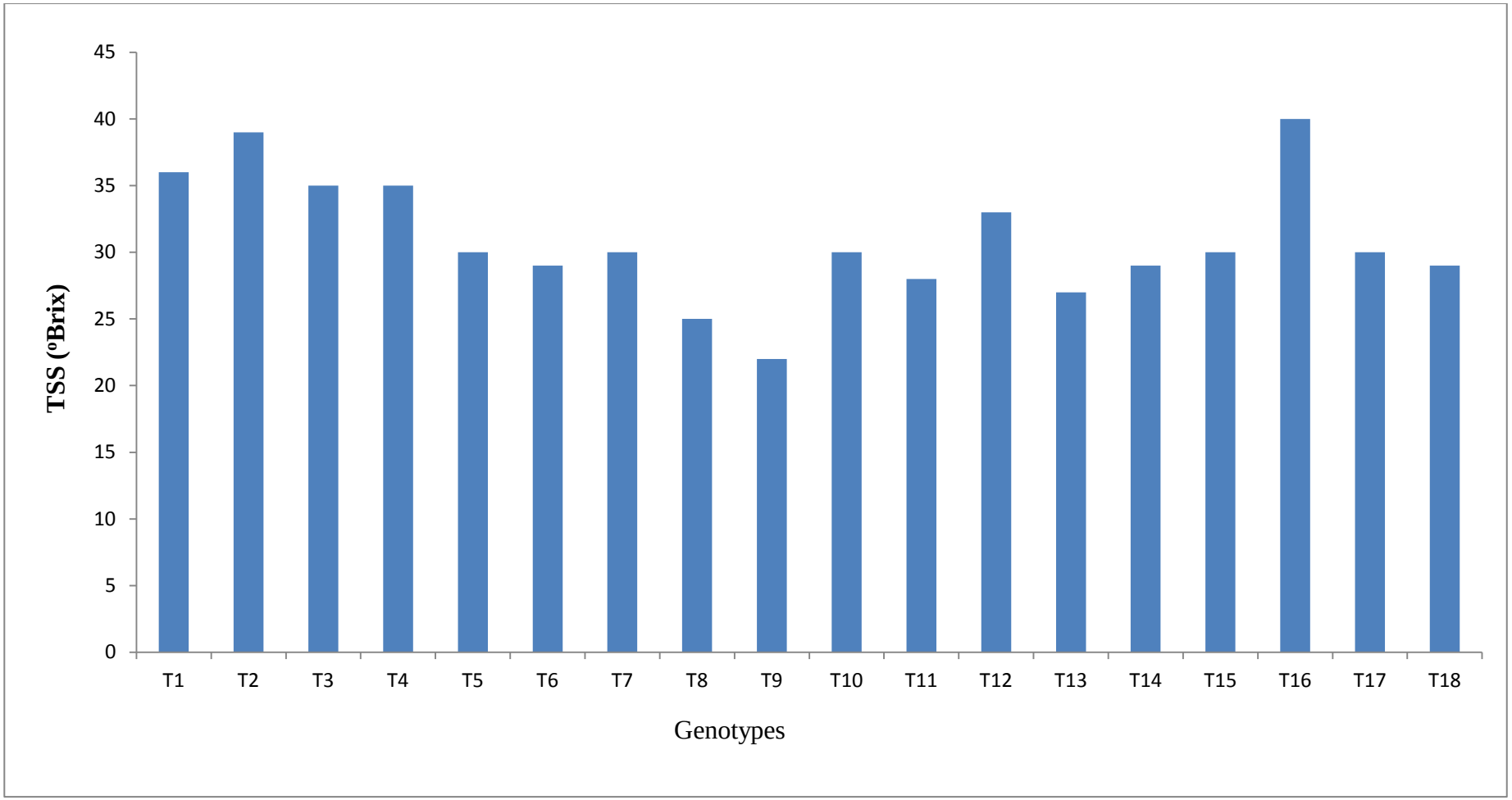


Fig.4i. TSS content in diverse genotypes of Bael

Table 4. 11: Acitivity of PAL enzyme in diverse genotypes of Bael

Treatments	PAL(µmole TCA/mg fw)
T_1 : (Pant Bael-1)	0.264
T_2 : (Pant Bael-2)	0.270
T_3 : (Pant Bael-3)	0.262
T_4 : (Pant Bael-4)	0.279
T_5 : (Pant Shivani)	0.294
T_6: (Pant Urvashi)	0.275
T_7 : (Haldi Nurmohamd)	0.279
T_8 : (Patharchatta-1)	0.259
T_9 : (Faizabad No.9)	0.270
T_{10} : (Faizabad Local)	0.270
T_{11} : (Pant Aparna)	0.314
T_{12} : (Pant Bael-10)	0.260
T_{13} : (Pant Sujata)	0.301
T_{14} : (Pant Bael-13)	0.266
T_{15} : (Pant Bael-14)	0.273
T_{16} : (Pant Bael- 15)	0.270
T_{17} : (Pant Bael -16)	0.266
T_{18} : (Gonda No.2)	0.275
S.Em. ±	0.0006
C.D. at 5%	0.0021

Table 4.12: Acitivity of PPO enzyme in diverse genotypes of Bael

Treatments	**PPO (ΔO.D./min/g fw)**
T_1 : (Pant Bael-1)	15.90
T_2 : (Pant Bael-2)	14.60
T_3 : (Pant Bael-3)	23.10
T_4 : (Pant Bael-4)	37.60
T_5 : (Pant Shivani)	22.70
T_6 : (Pant Urvashi)	15.80
T_7 : (Haldi Nurmohamd)	13.60
T_8 : (Patharchatta-1)	19.90
T_9 : (Faizabad No.9)	33.60
T_{10} : (Faizabad Local)	22.50
T_{11} : (Pant Aparna)	14.50
T_{12} : (Pant Bael-10)	15.70
T_{13} : (Pant Sujata)	15.90
T_{14} : (Pant Bael-13)	27.60
T_{15} : (Pant Bael-14)	33.60
T_{16} : (Pant Bael- 15)	17.50
T_{17} : (Pant Bael -16)	14.80
T_{18} : (Gonda No.2)	27.10
S.Em. ±	0.58
C.D. at 5%	1.66

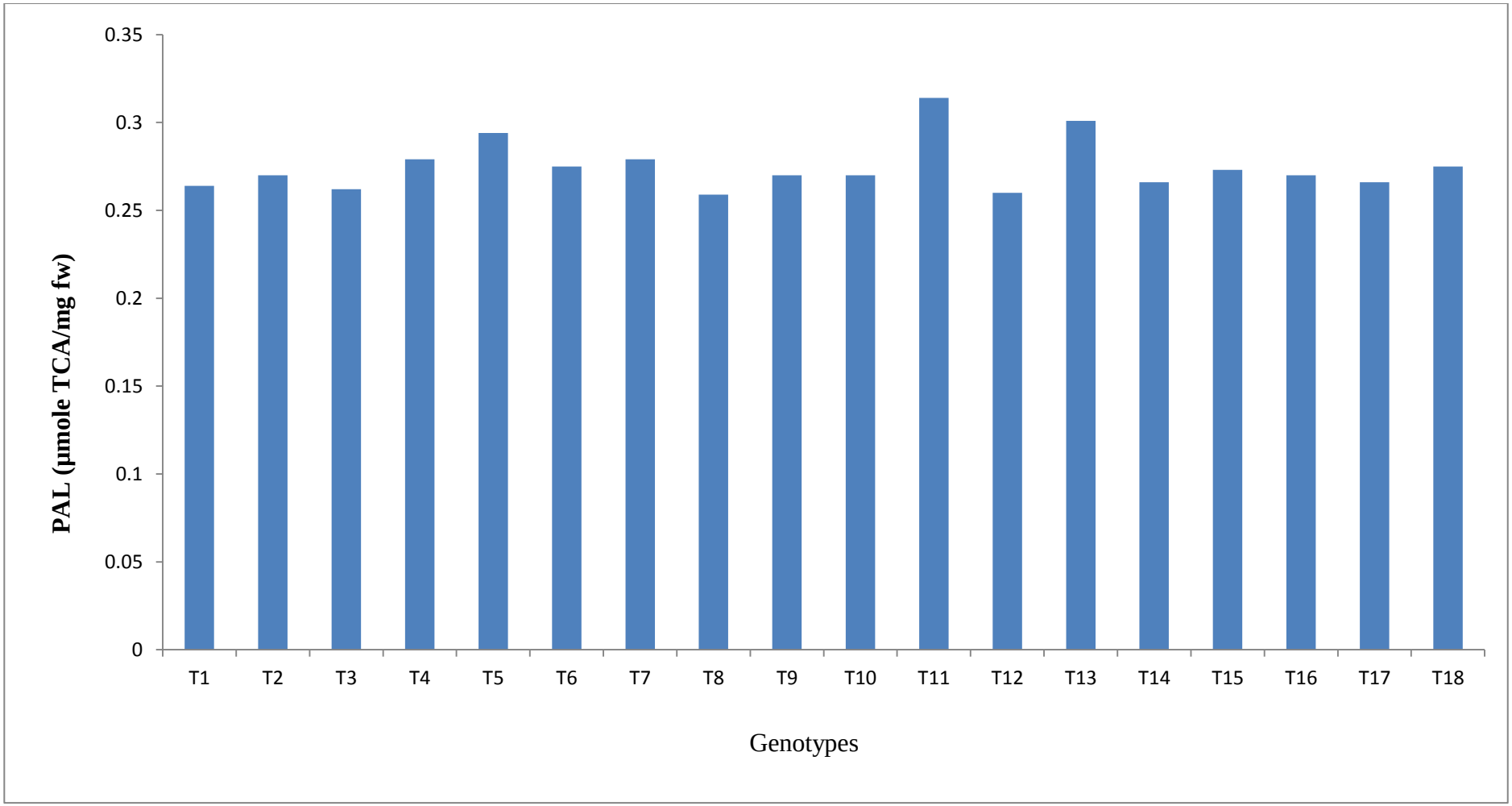


Fig.4j. Acitivity of PAL enzyme in diverse genotypes of Bael

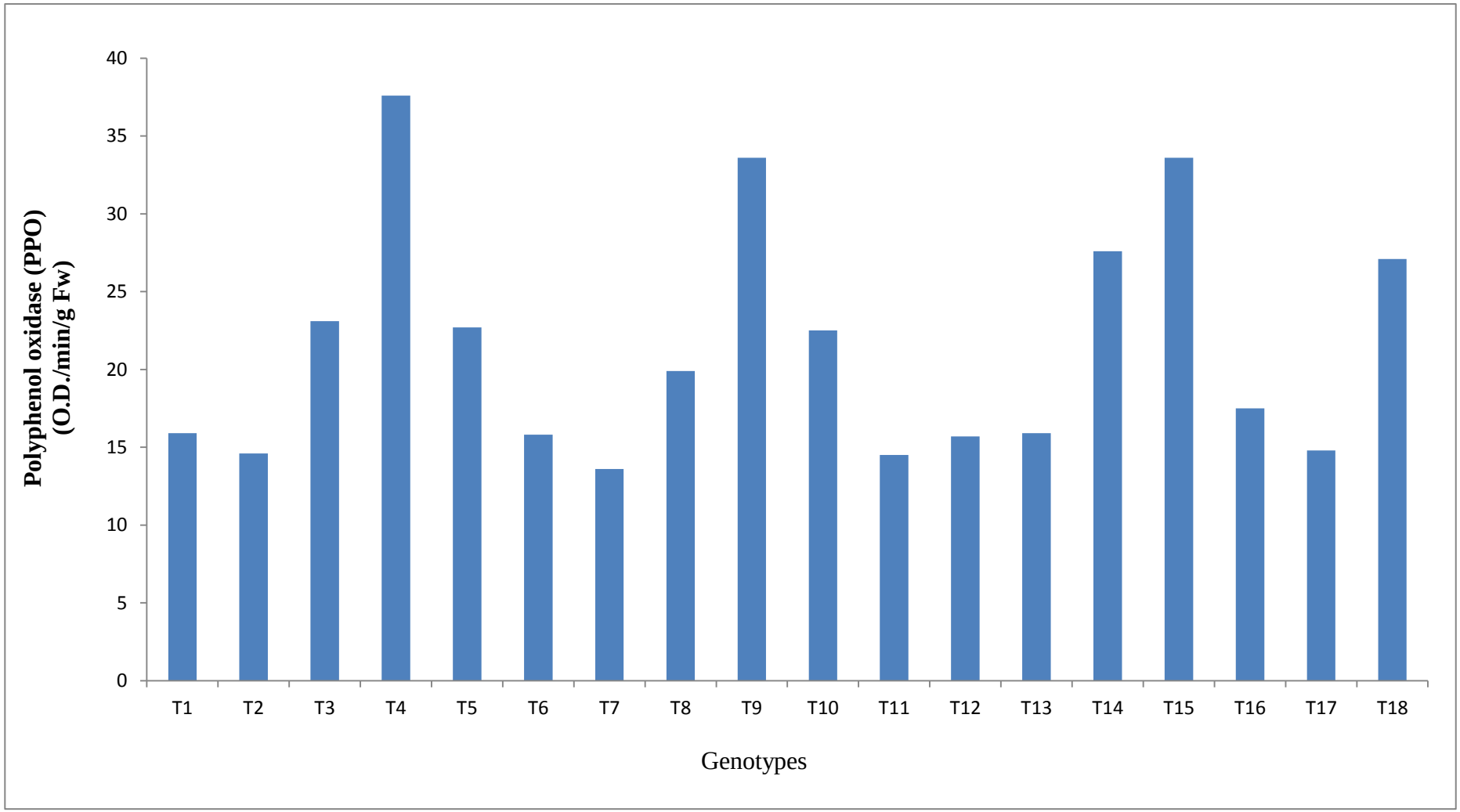


Fig.4k. Acitivityof PPO enzyme in diverse genotypes of Bael

(0.301 µmole TCA/mg Fw) and Pant Shivani (0.294 µmole TCA/mg Fw). Whereas minimum phenylalanine ammonia lyase enzyme acitivity reported from Patharchatta-1 (0.258 µmole TCA/mg Fw). Similar effect of genotype on phenylalanine ammonia lyase enzyme acitivity was reported by Lister *et al.* (1996) in apple and Fabiane *et al.* (2017) in peach genotypes.

4.1.4.3. Polyphenol oxidase (PPO)

The perusal of data in Table 4.12 and Fig.4k.clearly indicates that polyphenol oxidase enzyme activity significantly varies among various genotypes of Bael. The minimum value was noted in Haldi Nurmohamd (13.60 O.D./ min/g Fw). Whereas, the maximum value of polyphenol oxidase enzyme activity was found in Pant Bael -4 (37.60 O.D./ min/g Fw). The Polyphenol oxidase (PPO) is a copper-containing enzyme which is present in all plants. This enzyme catalyzes the oxidation of phenolic compounds to form corresponding quinone intermediates which polymerize to form an undesirable pigment. Polyphenols oxidase have received increasing attention because of their participation in bothnon-enzymic and enzymic browning reactions. Similar effect of genotypes on polyphenol oxidase activity reported by Joshi *et al.* (2007) and Kolodziejczyk *et al.* (2010) in apple.

4.2. Molecular Characterization

4.2.1. Simple Sequence Repeat (SSR) Analysis

In the present study, for the characterization of Bael genotype the microsatellite (SSR) markers were used with twenty four Bael genotypes. The 40 primers of citrus based SSR were selected from the review of literature. Out of 40 SSR primers, 31 primers amplified the DNA in Bael (Table 4.13).

4.2.1.1. Polymorphism Pattern of SSR

All the 31 SSRs primers amplified total number of 59 bands. The SSR marker CAC33 produced maximum number of 5 bands, while AC01, CAC23, CAGG09, CCSM147, CT01, CT21, CY48, GT03, SCM05, TAA1, TAA27 and TC26 produced minimum number of 1 band. The all 59 bands are polymorphic. The amplified fragments ranged from 100-700 bp. The per cent polymorphism obtained for SSR primers were 100 per cent. The polymorphic information content (PIC) was calculated for each primers and it was ranged from 0.234-0.998. The highest polymorphic information content (PIC) was calculated from CAC23 (0.998) and lowest polymorphic information content (PIC) was calculated from SCM05 (0.234).

S. N.	Specification	Particular
1	Total number of primer tested	40
2	Number of polymorphic primers	31
3	Number of monomorphic primers	0
6	Total number of polymorphic bands identified	59
7	Total number of monomorphic bands	0
8	Total number of bands	59
9	Size range of amplified products (bp)	100-800
10	Percent polymorphism	100
11	PIC value	0.234-0.998

The agarose gel electrophoresis was used to separate amplified product of SSR primers. The performance of individual primer to amplify genomic DNA of 24 genotypes is discussed as under.

AC01

This primer paired revealed two amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 600-800 bp. This primer pair gave PIC value of 0.889% and per cent polymorphism was 100 per cent with and Heterozygosity value 0.444.

AG14

This primer paired gave one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 100-200bp. The primer pair gave PIC value of 0.679, Heterozygosity value 0.549 and showed 100 per cent polymorphism.

CAC15

Primer generated two amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 150-200bp. The primer pair revealed PIC value of 0.941, Heterozygosity value 0.597 and 100 per cent polymorphism.

Table 4.13 : Level of polymorphism revealed by thirty one SSR primers in twenty four genotypes of Bael

S.No.	Primers	Amplifieed product	Number of Amplified alleles	Polymorphic band (s)	Monomorphic band (s)	Percent Polymorphism	PIC value
1	**AC01**	600-800	2	2	0	100	0.889
2	**AG14**	100-200	1	1	0	100	0.679
3	**CAC15**	150-200	2	2	0	100	0.941
4	**CAC23**	200-300	1	1	0	100	0.998
5	**CAC33**	200-600	5	5	0	100	0.986
6	**CAC39**	150-600	2	2	0	100	0.649
7	**CAT01**	150-650	2	2	0	100	0.760
8	**CAG01**	100-300	2	2	0	100	0.774
9	**CAGG9**	100-200	1	1	0	100	0.889
10	**CCSM13**	200-700	3	3	0	100	0.949
11	**CCSM18**	600-700	2	2	0	100	0.798
12	**CCSM147**	100-200	1	1	0	100	0.845
13	**CT02**	100-200	1	1	0	100	0.750
14	**CTT01**	200-800	3	3	0	100	0.851
15	**CCT01**	400-500	2	2	0	100	0.734
16	**CT21**	100-200	1	1	0	100	0.957
17	**CY01**	200-600	1	1	0	100	0.984
18	**CY05**	200-300	2	2	0	100	0.983
19	**CY19**	300-600	3	3	0	100	0.957
20	**CY23**	400-700	4	4	0	100	0.918
21	**CY37**	300-400	2	2	0	100	0.705
22	**CY48**	350-400	1	1	0	100	0.707
23	**CY51**	300-600	2	2	0	100	0.983
24	**GT03**	100-200	1	1	0	100	0.889
25	**SCM01**	100-200	2	2	0	100	0.964
26	**SCM05**	150-200	1	1	0	100	0.234
27	**TAA1**	150-200	1	1	0	100	0.438
28	**TAA15**	150-200	3	3	0	100	0.749
29	**TAA27**	200-700	1	1	0	100	0.373
30	**TAA41**	600-700	2	2	0	100	0.993
31	**TC26**	100-200	1	1	0	100	0.306

CAC23

This primer generated one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 200-300bp. This primer pair gave PIC value of 0.998, Heterozygosity value 0.080 and per cent polymorphism was 100 per cent.

CAC33

Primer CAC33 amplified five amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 200-600bp. This primer pair showed PIC value of 0.998, Heterozygosity value 0.920 and revealed 100 per cent polymorphism.

CAC39

This primer paired revealed two amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 150-600bp. The primer pair gave PIC value of 0.649, Heterozygosity value 0.431 and per cent polymorphism was 100 per cent.

CAT01

Primer generated two amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 150-600bp. The primer pair gave PIC value of 0.760, Heterozygosity value 0.955 and 100 per cent polymorphism.

CAG01

In Bael genotype, 2 alleles were amplified by primer CAG01 and produced 100 per cent polymorphic band.PIC of the primer was 0.774, Heterozygosity value 0.597 and size of SSR amplicons ranged from 100-300bp.

CAGG9

This primer generated one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 100-200bp. This primer pair gave PIC value of 0.889, Heterozygosity value 0.444 and exhibited 100 per cent polymorphism.

CCSM13

Primer generated three amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 200-700bp. The primer pair gave PIC value of 0.949, Heterozygosity value 1.472 and per cent polymorphism was 100 per cent.

CCSM18

This primer paired revealed two amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 600-700 bp. The primer pair gave PIC value of 0.798, Heterozygosity value 0.941 and showed 100 per cent polymorphism.

CCSM147

Primer CCSM147 revealed one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 100-200bp. The primer pair gave PIC value of 0.845, Heterozygosity value 0.444 and 100 per cent polymorphism.

CT02

In Bael genotypes one allele were amplified by primer CT02 on agrose gel electrophoresis. The size of SSR amplicons ranged from 100-200bp. The primer pair gave PIC value of 0.750, Heterozygosity value 0.500 and showed 100 per cent polymorphism.

CTT01

This primer generated three amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 200-800 bp. This primer pair gave PIC value of 0.851, Heterozygosity value 1.236 and per cent polymorphism was 100 per cent.

CCT01

Primer generated two amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 400-500bp. The primer pair gave PIC value of 0.734, Heterozygosity value 0.938 and per cent polymorphism was 100 percent.

CT21

On agarose this primer pair revealed one amplified SSR loci. The size of SSR amplicons ranged from 100-200bp. The primer pair gave PIC value of 0.957, Heterozygosity value 0.330 and cent per cent polymorphism.

CY01

This primer paired revealed one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 200-600 bp. This primer pair gave PIC value of 0.984, Heterozygosity value 0.219 and per cent polymorphism was 100 per cent.

CY05

Primer generated two amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 200-300 bp. The primer pair revealed PIC value of 0.983, Heterozygosity value 0.431 and 100 per cent polymorphism.

CY19

Primer CY19 amplified three amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 300-600 bp. This primer pair showed PIC value of 0.957, Heterozygosity value 0.743 and revealed 100 per cent polymorphism.

CY23

In Bael genotypes four allele were amplified by primer CT02 on agrose gel electrophoresis. The size of SSR amplicons ranged from 400-700 bp. The primer pair gave PIC value of 0.918, Heterozygosity value 1.514 and showed 100 per cent polymorphism.

CY37

This primer generated two amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 300-400 bp. This primer pair gave PIC value of 0.705, Heterozygosity value 1.150 and per cent polymorphism was 100 per cent.

CY48

Primer generated oneamplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 350-400 bp. The primer pair gave PIC value of 0.707, Heterozygosity value 0.497 and per cent polymorphism was 100 per cent.

CY51

On agarose this primer pair revealed two amplified SSR loci. The size of SSR amplicons ranged from 300-600bp. The primer pair gave PIC value of 0.983, Heterozygosity value 0.431 and cent per cent polymorphism.

GT03

This primer paired revealed one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 100-200 bp. This primer pair gave PIC value of 0.889, Heterozygosity value 0.444 and per cent polymorphism was 100 per cent.

Table 4.14 :Pair wise Jaccard's similarity coefficient among twenty four genotypes of Bael

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	
L1	1.000																								
L2	0.644	1.000																							
L3	0.712	0.458	1.000																						
L4	0.814	0.695	0.729	1.000																					
L5	0.881	0.593	0.797	0.831	1.000																				
L6	0.915	0.593	0.695	0.763	0.797	1.000																			
L7	0.898	0.610	0.712	0.780	0.814	0.949	1.000																		
L8	0.797	0.610	0.678	0.712	0.746	0.746	0.797	1.000																	
L9	0.322	0.475	0.508	0.373	0.373	0.305	0.288	0.492	1.000																
L10	0.305	0.559	0.492	0.390	0.356	0.288	0.271	0.441	0.814	1.000															
L11	0.695	0.712	0.712	0.712	0.712	0.644	0.661	0.661	0.627	0.610	1.000														
L12	0.729	0.610	0.644	0.678	0.746	0.712	0.729	0.695	0.559	0.508	0.797	1.000													
L13	0.831	0.576	0.712	0.746	0.780	0.881	0.898	0.729	0.356	0.339	0.661	0.763	1.000												
L14	0.627	0.712	0.610	0.678	0.644	0.576	0.593	0.695	0.695	0.644	0.831	0.695	0.627	1.000											
L15	0.508	0.661	0.559	0.525	0.525	0.458	0.475	0.508	0.712	0.729	0.780	0.644	0.542	0.746	1.000										
L16	0.559	0.644	0.508	0.576	0.576	0.508	0.525	0.559	0.593	0.542	0.729	0.627	0.559	0.661	0.780	1.000									
L17	0.780	0.627	0.661	0.763	0.797	0.729	0.746	0.746	0.441	0.458	0.746	0.746	0.712	0.678	0.627	0.746	1.000								
L18	0.814	0.593	0.729	0.797	0.797	0.831	0.814	0.712	0.441	0.390	0.746	0.780	0.814	0.678	0.593	0.644	0.797	1.000							
L19	0.695	0.678	0.678	0.712	0.712	0.644	0.661	0.661	0.593	0.542	0.864	0.763	0.661	0.729	0.746	0.763	0.746	0.814	1.000						
L20	0.695	0.712	0.678	0.712	0.712	0.644	0.661	0.661	0.593	0.576	0.864	0.763	0.661	0.729	0.780	0.763	0.746	0.780	0.966	1.000					
L21	0.898	0.610	0.780	0.814	0.847	0.881	0.932	0.797	0.322	0.305	0.695	0.763	0.864	0.627	0.508	0.559	0.780	0.847	0.695	0.695	1.000				
L22	0.678	0.695	0.695	0.695	0.729	0.627	0.644	0.644	0.610	0.593	0.915	0.780	0.644	0.780	0.763	0.712	0.729	0.729	0.881	0.881	0.678	1.000			
L23	0.458	0.678	0.576	0.508	0.508	0.407	0.424	0.559	0.797	0.780	0.729	0.525	0.390	0.729	0.780	0.695	0.610	0.508	0.695	0.695	0.458	0.712	1.000		
L24	0.373	0.627	0.492	0.424	0.424	0.322	0.339	0.475	0.746	0.797	0.678	0.508	0.373	0.644	0.797	0.644	0.525	0.458	0.644	0.678	0.373	0.695	0.881	1.000	
	16.627	14.729	14.644	14.186	13.593	12.153	11.458	11.034	9.898	8.814	11.034	9.356	7.847	8.000	7.373	6.525	5.932	5.136	4.881	3.949	2.508	2.407	1.881	8.540	1.000

Table 4.15. Genetic parameters (Gene diversity & Heterozygosity estimated for 31 SSR primers in 24 genotypes of Bael.

S.No.	Primers	Gene diversity	Heterozygosity
1.	**AC01**	0.019	0.444
2.	**AG14**	0.023	0.549
3.	**CAC15**	0.025	0.597
4.	**CAC23**	0.003	0.080
5.	**CAC33**	0.038	0.920
6.	**CAC39**	0.018	0.431
7.	**CAT01**	0.040	0.955
8.	**CAG01**	0.025	0.597
9.	**CAGG9**	0.019	0.444
10.	**CCSM13**	0.061	1.472
11.	**CCSM18**	0.039	0.941
12.	**CCSM147**	0.019	0.444
13.	**CT02**	0.021	0.500
14.	**CTT01**	0.052	1.236
15.	**CCT01**	0.039	0.938
16.	**CT21**	0.014	0.330
17.	**CY01**	0.009	0.219
18.	**CY05**	0.018	0.431
19.	**CY19**	0.031	0.743
20.	**CY23**	0.063	1.514
21.	**CY37**	0.048	1.149
22.	**CY48**	0.021	0.497
23.	**CY51**	0.018	0.431
24.	**GT03**	0.019	0.444
25.	**SCM01**	0.025	0.608
26.	**SCM05**	0.009	0.219
27.	**TAA1**	0.016	0.375
28.	**TAA15**	0.062	1.493
29.	**TAA27**	0.014	0.330
30.	**TAA41**	0.013	0.306

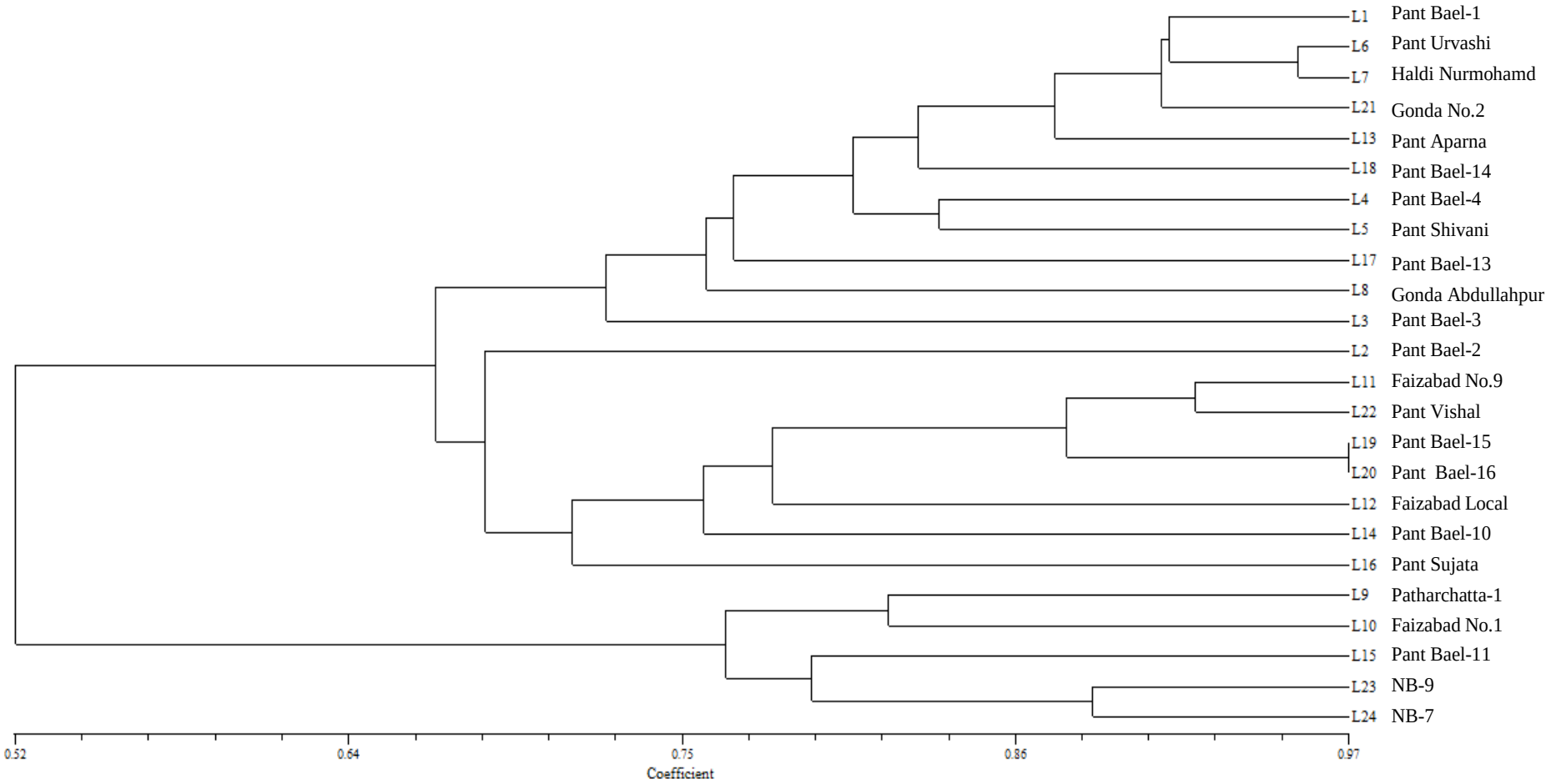


Fig. 4l: Dendrogram illustrating the phylogenetic relationship among 24 Bael genotypes based on UPGMA cluster analysis

SCM01

Primer generated two amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from100-200 bp. The primer pair revealed PIC value of 0.964,Heterozygosity value 0.608 and 100 per cent polymorphism.

SCM05

This primer generated one amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 150-200bp. This primer pair gave PIC value of 0.234 Heterozygosity value 0.219 and per cent polymorphism was 100 per cent.

TAA1

In Bael genotypes one allele were amplified by primer CT02 on agrose gel electrophoresis. The size of SSR amplicons ranged from 150-200bp. The primer pair gave PIC value of 0.438, Heterozygosity value 0.375 and showed 100 per cent polymorphism.

TAA15

Primer TAA15 revealed three amplified SSR loci on agrose gel electrophoresis. The size of SSR amplicons ranged from 150-200bp. The primer pair gave PIC value of 0.749, Heterozygosity value 1.493 and 100 per cent polymorphism.

TAA27

Primer generated one amplified loci on agrose gel electrophoresis. The size of SSR amplicons ranged from200-700 bp. The primer pair revealed PIC value of 0.373, Heterozygosity value 0.330 and 100 per cent polymorphism.

TAA41

On agarose this primer pair revealed two amplified SSR loci. The size of SSR amplicons ranged from 600-700bp. The primer pair gave PIC value of 0.993, Heterozygosity value 0.306 and cent per cent polymorphism.

TC26

On agarose this primer pair revealed one amplified SSR loci. The size of SSR amplicons ranged from 100-200bp. The primer pair gave PIC value of 0.306, Heterozygosity value 0.278 and cent per cent polymorphism.

4.3.3. Genetic diversity analysis using SSR primers

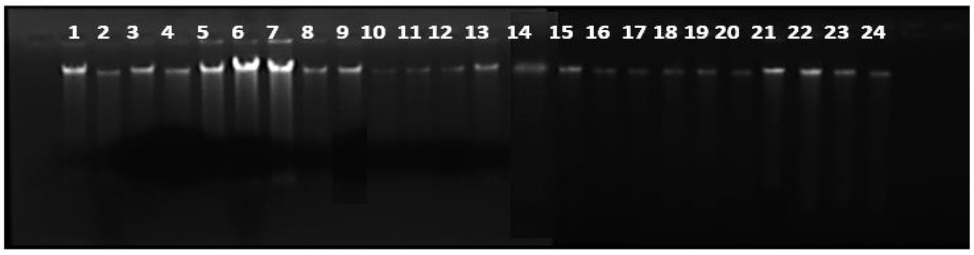
Data scored on 24 Bael genotypes with 31 microsatellite (SSR) primers were used to generate Jaccard's similarity coefficient presented in **Table4.14**. In Bael genotypes Jaccard's similarity coefficient varied from 0.271 to 0.951 with an average value of 0.692. Pant Bael-1 and Pant Urvashi (0.915) were found to be the most similar genotypes among the 24 genotypes studied followed by Pant Bael-1 and Haldi Nurmohmad (0.898),Haldi Nurmohmad and Pant Aparna (0.898). Minimum Jaccard's similarity coefficient was found in Haldi Nurmohmad with Faizabad No.1 (0.271). Similar findings were also reported by other researchers in Bael by Nayak *et al.*(2013), Mujeeb*et al.* (2017) and Misra and Singh, (2018).

4.3.4. Cluster analysis based on SSR markers

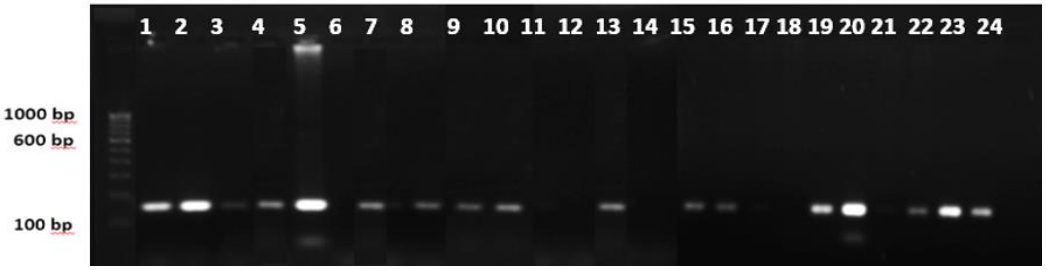
The phylogenetic tree was constructed through NTSYSpc cluster analysis software using UPGMA (Un-weighted pair group method with arithmetic mean). All 24 genotype were demarcated into two major clusters A and B (Fig.4l) at 52 per cent similarity. The major cluster A consist of 19 genotypes, which was further divided into two sub clusters IA and IIA at 66 per cent similarity. The eleven genotypes of Bael were separated into sub cluster IA and eight genotypes in subcluster at IIA. The sub cluster IA again bifurcated into small cluster IA1 and IA2 at 72 per cent similarity. The small cluster IA1 consist of 8 genotypes and small cluster IA2 consist of single genotype Pant Bael -3. The sub cluster IIA again bifurcated into small cluster IIA1 and IIA2 at 68 per cent similarity. The small cluster IIA1 consist of single genotype Pant Bael-2 and rest seven genotype comes under small cluster IIA2.The IIA2 again divided into super small cluster IIA2a and IIA2b at 70 per cent similarity which consist of six genotypes and single genotype Pant Sujata respectively. The major cluster B consist of 5 genotypes, which was further divided into two sub cluster IB and IIB at 78 per cent similarity. The sub cluster IB consist of two genotypes Patharchatta -1 and Faizabad No.1. However the, sub cluster IIB consist of genotypes three genotypes *i.e.*, Pant Bael-11, Narendera Bael-7 and Narendra Bael-9.

PLATE 1

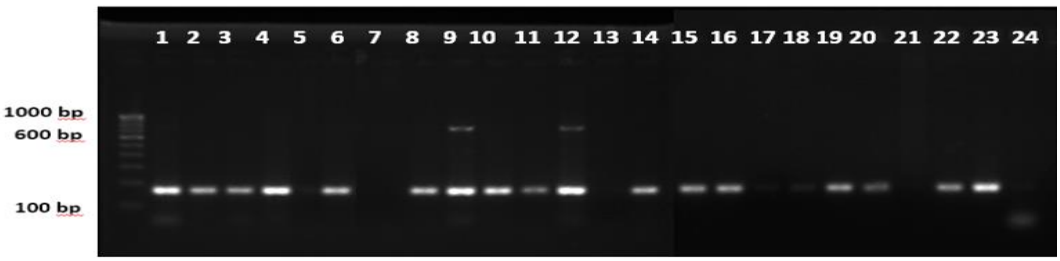
Electrophoretic banding pattern of genomic DNA from 24 Bael genotypes on 0.8 % agarose gel.



Amplification pattern of AG14

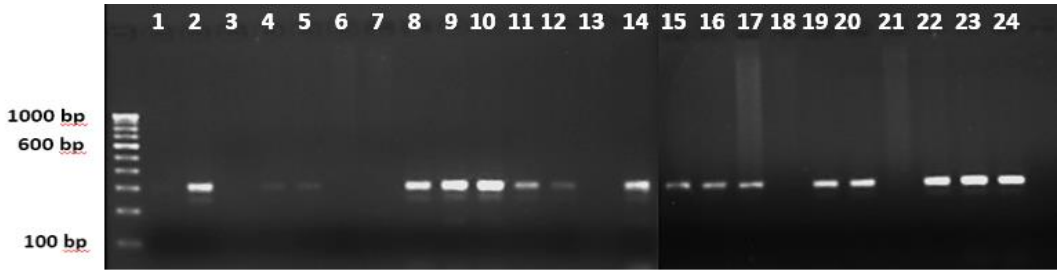


Amplification pattern of CAC39

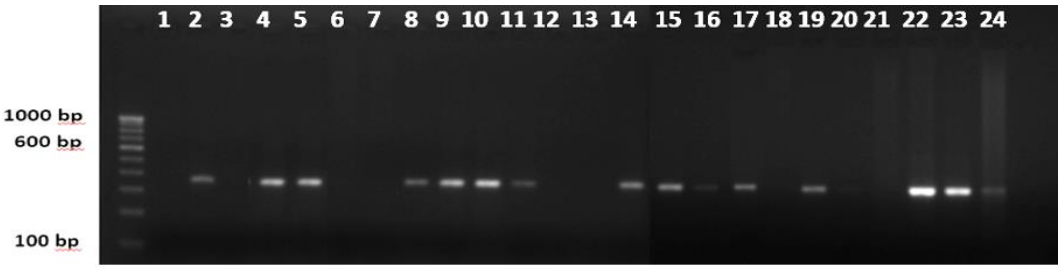


Amplification pattern of CTT01

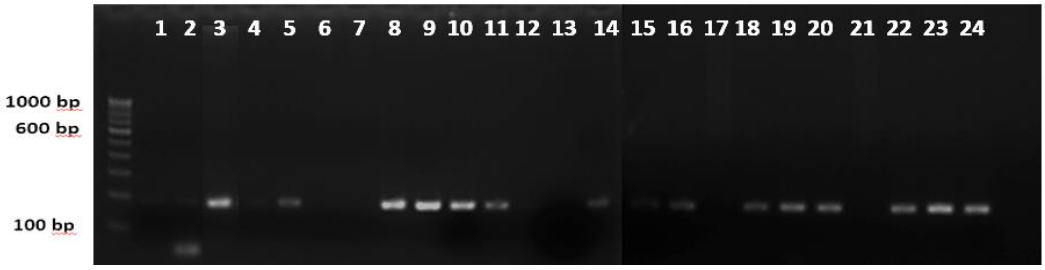
Amplification pattern of CY37



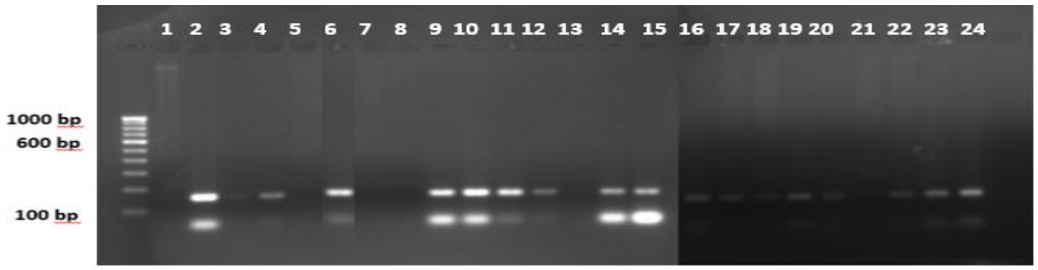
Amplification pattern of CY 48



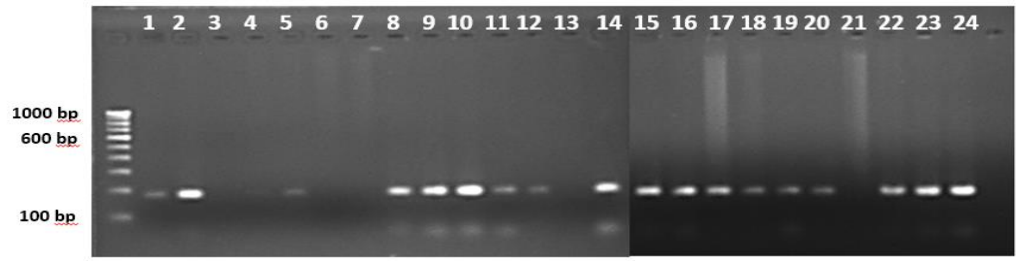
Amplification pattern of SCM05



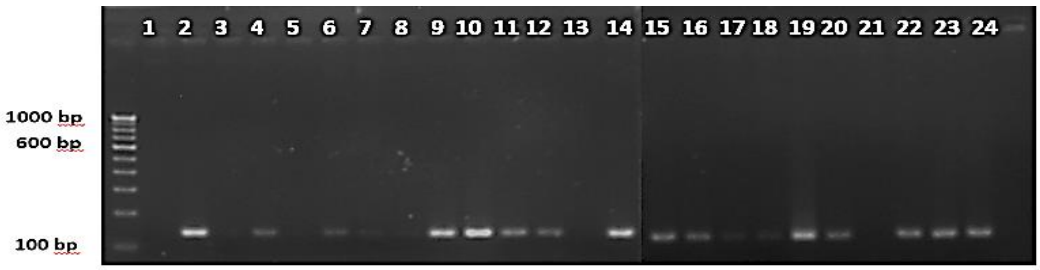
Amplification pattern of TAA1



Amplification pattern of TAA27



Amplification pattern of TC-26



4.3. Development of appetizer from diverse genotypes of Bael.

Today raising health problems are great issue of concern. The unhealthy food habits pose unique challenges to human health. The nutrition deprived diet system leads weak health and feeble immunity system which makes people prone to diseases and many disorders. Ultimately this all results as raising health problems like heart diseases, diabetes and cancers. To fight against all these problem nutraceutical rich diet can play significant role. The novel concept of functional foods emerging as great solution for several health issues.A functional food also called as Foods for Specified Health Use (FOSHU). It refers a food given an additional function by adding new ingredients or more of existing ingredients. The functional foods may be designed to have physiological benefits and its also reduce the risk of chronic diseases beyond basic nutritional functions, and may be similar in appearance to conventional food and consumed as part of a regular diet.Therefore, an effort was made to develop an appetizer by adding new ingredients to Bael powder with nutraceutical properties.

4.3.1. Quality analysis

4.3.1.1. Total sugar

The effect of genotypes and ingredients on total sugar of appetizer are presented in Table 4.17 and discussed under following subheading.

4.3.1.1.1. Effect of genotypes on Total sugar

The effect of genotypes on total sugar of appetizer was found to be significant. The maximum total sugar was obtained in appetizer prepared from Pant Bael-16 (14.297%) followed by Pant Bael-4(14.092%). Whereas, the minimum total sugar was obtained from appetizer prepared from Gonda No.2 (8.77%). This might be due the genetic attribute of genotype. Similar effect of genotypes on total sugar of fruit based value added product also reported by Choudhary *et al.* (2008) in guava nectar and Pawar and Patil (2013) in aonla candy.

4.3.1.1.2. Effect of ingredients on Total sugar

An effect of ingredients on total sugar of appetizers was also found significant. Maximum value of total sugar was obtained from ingredients B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). However, the minimum

Table 4.16: List of medicinal plant species used as ingredient for preparation of appetizers

S.No.	Ingredients	Part used	Health benefits	Refrences
1	Bael **Rutaceae** *(Aegle marmelos* Correa)	Unripe fruit	Fruit possesses broad range of therapeutic effects that includes free radical scavenging, antioxidant, inhibition of lipid peroxidation, antibacterial, antiviral, anti-diarrheal, gastroprotective, anti-ulcerative colitis, hepatoprotective, antidiabetic, cardioprotective and radioprotective effects.	Baliga *et al.* (2012)
2	Fenugreek **Fabaceae** (Trigonella foenum-graecum)	Seed	Hypoglycemic, Hypocholesterolaemic, effects and Anti-inflammatory effects.	Moradi kor *et al.* (2013)
3	Aonla **Euphorbiaceae** *(Emblica officinalis* Gaertn.)	Fruit	Antimicrobial, antioxidant, anti-inflammatory, analgesic and antipyretic, adaptogenic, hepatoprotective, antitumor and antiulcerogenic	Gaire and Subedi (2015)
4	Jamun **Myrtaceae** *(Syzygium cumini* (L.) Skeels.)	Seed	Seeds are antidiabetic, used in treatment of mouth, throat, intestines and genitourinary tract ulcers	Ayyanar and Babu (2012)
5	Black pepper **Piperaceae** (Piper nigrum)	Seed	It is a natural antioxidant. It acts as antiinflammatory, anticancer, antiperiodic and antipyretic.	Meghwal and Goswami (2012)

Table 4.17: Effect of genotypes and ingredients on total sugar of appetizer

Treatments	Total Sugar (%)						
	Ingreditents						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1(Pant Bael-1)	10.300 (3.360)	10.100 (3.330)	5.400 (2.525)	12.300 (3.645)	6.700 (2.771)	11.010 (3.464)	9.302 (3.183)
A_2(Pant Bael-2)	13.450 (3.800)	13.420 (3.796)	7.340 (2.885)	16.450 (4.176)	7.450 (2.904)	14.560 (3.943)	12.112 (3.584)
A_3(Pant Bael-3)	11.630 (3.552)	11.340 (3.511)	6.560 (2.746)	12.890 (3.726)	7.010 (2.827)	12.650 (3.693)	10.347 (3.343)
A_4(Pant Bael-4)	16.340 (4.163)	16.310 (4.160)	8.370 (3.059)	19.430 (4.519)	8.430 (3.068)	15.670 (4.082)	14.092 (3.842)
A_5(Pant Shivani)	11.030 (3.467)	11.010 (3.464)	6.310 (2.700)	12.350 (3.652)	7.450 (2.904)	13.050 (3.747)	10.200 (3.322)
A_6(Pant Aparna)	12.330 (3.650)	12.290 (3.644)	6.260 (2.691)	13.450 (3.800)	7.280 (2.874)	14.380 (3.921)	10.998 (3.430)
A_7(Pant Bael-10)	14.430 (3.927)	14.410 (3.924)	8.450 (3.072)	20.560 (4.643)	8.320 (3.050)	16.030 (4.126)	13.700 (3.790)
A_8 (Pant Bael-14)	12.010 (3.605)	12.010 (3.605)	9.010 (3.162)	21.540 (4.747)	8.910 (3.146)	14.080 (3.882)	12.927 (3.691)
A_9(Pant Bael-15)	10.490 (3.388)	10.400 (3.375)	5.120 (2.469)	12.450 (3.666)	6.010 (2.644)	13.480 (3.804)	9.658 (3.224)
A_{10}(Pant Bael-16)	15.090 (4.010)	15.010 (4.000)	8.240 (3.037)	21.070 (4.697)	8.340 (3.054)	18.030 (4.361)	14.297 (3.860)
A_{11}(Gonda No.2)	9.420 (3.226)	9.430 (3.227)	4.500 (2.340)	12.320 (3.648)	5.600 (2.565)	11.340 (3.511)	8.768 (3.086)
Mean	12.411 (3.650)	12.339 (3.640)	6.869 (2.790)	15.892 (4.084)	7.409 (2.892)	14.025 (3.867)	
Interaction (A x B)							
S.Em. ±	(0.031)		(0.023)		(0.077)		
C.D. at 5%	(0.088)		(0.065)		(0.215)		

Ingredients detail:

B_1: 100% Bael powder,
B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,
B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,
B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**
B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**
B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

(Figures shown in parenthesis are square root transformed value)

value of total sugar was obtained from B_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper). The different ingredients used for appetizer preparation was varying in their total sugar content which affects the total sugar of appetizer. The effect of ingredients on total sugar of other value added products also reported by Asghar *et. al.* (2016) functional Bael jam and Chauhan *et al.* 2016 in Bael vermouth.

4.3.1.1.3. Interaction effect of genotypes and ingredients on Total sugar

The significant effect of genotypes and ingredients on Total sugar of appetizer was observed. The highest value of total sugar was obtained fromA_8B_4 (Pant Bael-14 with 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper)which was found at par with A_7B_4. Whereas the lowest sugar was obtained from $A_{11}B_3$ (Gonda No.2 with 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper).

4.3.1.2. Reducing sugar

An effect of genotypes and ingredients on reducing sugar of appetizer is presented in Table 4.18 and discussed under following subheading.

4.3.1.2.1. Effect of genotypes on reducing sugar

The effect of genotype on reducing sugar of appetizer was found to be significant.The maximum reducing sugar was obtained from Pant Bael -16(4.755%), found at par with Pant Bael- 4 (4.423%), whereas the minimum reducings was obtained from Pant Bael-1(2.25%). This might be due to the difference in genetic attributes of different genotype and the effect of drying temperature. Similar effect of genotype on reducing sugar of value added product was noted by Choudhary *et al.* (2008) and Pawar and Patil (2013).

4.3.1.2.2. Effect of ingredients on reducing sugar

A significant effect of ingredients on reducing sugar of appetizer was noted. The maximum reducing sugar (6.95%) was obtained from B_4 (% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). However, the minimum value of reducing sugar (1.68%) was obtained from B_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper). This might be due to the fact that varying

Table 4.18 : Effect of genotypes and ingredients on reducing sugar of appetizer

Treatments	Reducing Sugar (%)						
	Ingredients						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	2.280 (1.808)	2.070 (1.748)	1.170 (1.446)	4.870 (2.421)	2.160 (1.774)	0.950 (1.389)	2.250 (1.768)
A_2 :(Pant Bael-2)	4.320 (2.305)	3.930 (2.218)	2.220 (1.791)	7.140 (2.852)	1.810 (1.672)	3.890 (2.209)	3.885 (2.175)
A_3 :(Pant Bael-3)	2.840 (1.957)	2.690 (1.918)	1.550 (1.592)	5.330 (2.515)	0.640 (1.270)	4.350 (2.311)	2.900 (1.927)
A_4 :(Pant Bael-4)	3.850 (2.200)	3.880 (2.207)	1.340 (1.524)	9.390 (3.223)	2.420 (1.846)	5.660 (2.579)	4.423 (2.263)
A_5 :(Pant Shivani)	1.800 (1.669)	1.770 (1.660)	1.300 (1.511)	4.900 (2.428)	2.430 (1.849)	5.040 (2.456)	2.873 (1.929)
A_6 :(Pant Aparna)	2.240 (1.796)	2.280 (1.808)	1.250 (1.494)	6.110 (2.665)	2.180 (1.780)	5.450 (2.538)	3.252 (2.013)
A_7 :(Pant Bael-10)	3.090 (2.020)	3.000 (1.997)	1.080 (1.435)	9.670 (3.266)	1.420 (1.550)	6.750 (2.783)	4.168 (2.175)
A_8 :(Pant Bael-14)	1.790 (1.666)	2.000 (1.728)	1.970 (1.719)	8.980 (3.158)	2.680 (1.915)	3.010 (2.000)	3.405 (2.031)
A_9 :(Pant Bael-15)	0.930 (1.381)	1.200 (1.477)	2.080 (1.751)	5.110 (2.470)	1.920 (1.705)	4.570 (2.358)	2.635 (1.857)
A_{10} :(Pant Bael-16)	4.060 (2.248)	4.000 (2.234)	3.230 (2.054)	10.020 (3.319)	2.090 (1.754)	5.130 (2.475)	4.755 (2.347)
A_{11} :(Gonda No.2)	1.920 (1.705)	1.980 (1.722)	1.270 (1.500)	4.870 (2.421)	2.160 (1.774)	4.110 (2.259)	2.718 (1.897)
Mean	2.647 (1.887)	2.618 (1.883)	1.678 (1.622)	6.945 (2.794)	1.992 (1.717)	4.446 (2.305)	
Interaction (A x B)							
	A		B		AxB		
S.Em. ±	(0.030)		(0.022)		(0.075)		
C.D. at 5%	(0.084)		(0.060)		(0.213)		

Ingredients detail:

B_1: 100% Bael powder,
B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,
B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,
B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**
B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**
B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

(Figures shown in parenthesis are square root transformed value)

reducing sugar composition of different ingredients. Same results on effect of ingredients on reducing sugar of value added product are also obtained by Asghar *et al.* (2016) in functional Bael jam and Chauhan *et al.* (2016) in Bael vermouth.

4.3.1.2.3. Interaction effect of genotypes and ingredients on reducing sugar

The interaction effect of genotypes and ingredients on reducing sugar was also found to be significant. Highest value of reducing sugar was noted in $A_{10}B_4$(Pant Bael-16 with 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper) which was found at par with A_7B_4, A_4B_4 and A_8B_4 Whereas, the minimum value was obtained from A_9B_1(Pant Bael-15 100% Bael powder).

4.3.1.3. Non reducing sugar

The effect of genotypes and ingredients on non reducing sugar of appetizer are presented in Table 4.19 and discussed under following subheading.

4.3.1.3.1. Effect of genotypes on non reducing sugar

An effect of genotypes on non reducing sugar of appetizer were found to be significant. The maximum value of non reducing sugar was found in Pant Bael-4 (9.67%) which was found at par withPant Bael-10 (9.532%) and Pant Bael -14 (9.522%). Whereas, the minimum value was noted in Gonda No.2 (6.05%). The variation in reducing sugar might be due to the differences in the genetic constitution of genotypes.

4.3.1.3.2. Effect of ingredients on non reducing sugar

Significant effect of ingredients on non reducing sugar of appetizer was noted. Highest value of non reducing sugar was noted from B_1 (100% Bael powder) which was found at par with B_2 (96% Bael powder + 3% Black salt + 1% Black pepper) and B_6 (24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper). This might be due to the fact that difference in non reducing sugar composition of various ingredients used for appetizer preparation.

4.3.1.3.3. Interaction effect of genotypes and ingredients on non reducing sugar

The interaction effect of genotypes and ingredients on non reducing sugar was also found to be significant. The maximum value obtained from $A_{10}B_6$ (Pant Bael-16

Table 4.19: Effect of genotypes and ingredients on non reducing sugar of appetizer

Treatments	Non reducing Sugar (%)						
	Ingredients						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	8.020 (3.000)	8.030 (3.002)	4.230 (2.280)	7.430 (2.900)	4.540 (2.347)	10.060 (3.323)	7.052 (2.809)
A_2 :(Pant Bael-2)	9.130 (3.180)	9.490 (3.236)	5.120 (2.468)	9.310 (3.208)	5.640 (2.572)	10.670 (3.414)	8.227 (3.013)
A_3 :(Pant Bael-3)	8.790 (3.126)	8.650 (3.104)	5.010 (2.446)	7.560 (2.922)	6.370 (2.711)	8.300 (3.047)	7.447 (2.893)
A_4 :(Pant Bael-4)	12.490 (3.671)	12.430 (3.663)	7.030 (2.830)	10.040 (3.320)	6.010 (2.643)	10.010 (3.316)	9.668 (3.241)
A_5 :(Pant Shivani)	9.230 (3.196)	9.240 (3.197)	5.010 (2.446)	7.450 (2.903)	5.020 (2.448)	8.010 (2.999)	7.327 (2.865)
A_6 :(Pant Aparna)	10.090 (3.328)	10.010 (3.316)	5.010 (2.446)	7.340 (2.884)	5.100 (2.464)	8.930 (3.149)	7.747 (2.931)
A_7 :(Pant Bael-10)	11.340 (3.511)	11.410 (3.521)	7.370 (2.890)	10.890 (3.446)	6.900 (2.807)	9.280 (3.204)	9.532 (3.230)
A_8 :(Pant Bael-14)	10.220 (3.347)	10.010 (3.316)	7.040 (2.832)	12.560 (3.681)	6.230 (2.685)	11.070 (3.472)	9.522 (3.222)
A_9 :(Pant Bael-15)	9.560 (3.247)	9.200 (3.191)	3.040 (2.000)	7.340 (2.884)	4.090 (2.249)	8.910 (3.145)	7.023 (2.786)
A_{10} :(Pant Bael-16)	11.030 (3.466)	11.010 (3.464)	5.010 (2.446)	11.050 (3.469)	6.250 (2.688)	12.900 (3.727)	9.542 (3.210)
A_{11} :(Gonda No.2)	7.500 (2.912)	7.450 (2.903)	3.230 (2.047)	7.450 (2.903)	3.440 (2.098)	7.230 (2.865)	6.050 (2.622)
Mean	9.764 (3.271)	9.721 (3.265)	5.191 (2.466)	8.947 (3.138)	5.417 (2.519)	9.579 (3.242)	

Interaction (A x B)

	A	B	AxB
S.Em. ±	(0.041)	(0.030)	(0.100)
C.D. at 5%	(0.115)	(0.085)	(0.281)

Ingredients detail:

B_1: 100% Bael powder,
B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,
B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,
B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**
B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**
B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

(Figures shown in parenthesis are square root transformed value)

with24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper which was also found at par with other treatment A_8B_4, A_4B_1, A_4B_2, A_7B_1, A_7B_2 and A_8B_6whereas the mnimimu value noted from A_9B_3 (Pant Bael-15 with48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper).

4.3.1.4. Titratable acidity

Acidity is an important attribute because it gives the characteristic sourness to the product. It also enhances the characteristic flavor and taste of appetizer. The organic acid, present in the fruit powder influenced the colour, flavour, taste and keeping quality. The effect of genotypes and ingredients on titratable acidity of appetizer is presented in Table 4.20 and discussed under following subheading.

4.3.1.4.1. Effect of genotypes on titratable acidity

The effect of genotypes on titratable acidity of appetizer was found to be significant. The maximum value 0.65% noted from Pant Bael-2 and Pant Bael-3 which was found at par with Gonda no.2, Pant Bael-10 and Pant Shivani. Whereas, the minimum value 0.33% found in Pant Bael-14.The differences in titratable acidity of appetizer from different genotype might be due to the fact that varying potential of genotype to synthesis the organic acid and also the effect of drying temperature.Similar effect of genotype on titratable acidity of value added product was reported by Jain and Nema (2007), Pawar and Patil (2013), Ratnasooriya *et al.* (2012) and Abhangrao *et al.* (2017).

4.3.1.4.2. Effect of ingredients on titratable acidity

An effect of on titratable acidity of appetizer was found to be significant. The maximum value of on titratable acidity 1.03% found in B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). Whereas, the minimum value of on titratable acidity was noted in B_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper). Each ingredient used in preparation of appetizer have its own organic acid compositionwhichaffect the titratable acidity of appetizer. Similar effect of ingredients on tritatable acidity of value added product of fruit was observed by Jain and Nema (2007), Elbelazi *et al.* (2015), Asghar *et. al.* (2016), Bhatt and Verma (2016), Chauhan *et al.* (2016), Abhangrao *et al.* (2017) and Bisen *et al.* (2017).

Table 4.20 : Effect of genotypes and ingredients on titratable acidity of appetizer

Treatments	Titratable acidity (%)						
	Ingredients						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	0.200 (0.450)	0.280 (0.530)	0.280 (0.530)	1.400 (1.180)	0.280 (0.530)	0.720 (0.850)	0.527 (0.678)
A_2 :(Pant Bael-2)	0.300 (0.550)	0.300 (0.550)	0.300 (0.550)	1.500 (1.220)	0.400 (0.630)	1.100 (1.050)	0.650 (0.773)
A_3 :(Pant Bael-3)	0.500 (0.710)	0.500 (0.710)	0.200 (0.450)	1.300 (1.140)	0.400 (0.630)	1.000 (1.000)	0.650 (0.773)
A_4 :(Pant Bael-4)	0.300 (0.550)	0.300 (0.550)	0.200 (0.450)	1.000 (1.000)	0.240 (0.490)	0.440 (0.660)	0.413 (0.617)
A_5 :(Pant Shivani)	0.520 (0.720)	0.600 (0.770)	0.400 (0.630)	0.900 (0.950)	0.240 (0.490)	0.990 (0.950)	0.593 (0.752)
A_6 :(Pant Aparna)	0.260 (0.510)	0.300 (0.550)	0.360 (0.600)	0.800 (0.890)	0.300 (0.550)	0.820 (0.910)	0.473 (0.668)
A_7 :(Pant Bael-10)	0.400 (0.630)	0.320 (0.570)	0.240 (0.490)	1.300 (1.140)	0.300 (0.550)	0.960 (0.980)	0.587 (0.727)
A_8 :(Pant Bael-14)	0.200 (0.450)	0.200 (0.450)	0.140 (0.370)	0.600 (0.770)	0.260 (0.510)	0.600 (0.770)	0.333 (0.553)
A_9 :(Pant Bael-15)	0.300 (0.550)	0.340 (0.580)	0.240 (0.490)	0.800 (0.890)	0.200 (0.450)	0.560 (0.750)	0.407 (0.618)
A_{10} :(Pant Bael-16)	0.500 (0.710)	0.400 (0.630)	0.100 (0.320)	0.700 (0.840)	0.400 (0.630)	0.640 (0.800)	0.457 (0.655)
A_{11} :(Gonda No.2)	0.500 (0.710)	0.500 (0.710)	0.320 (0.570)	1.000 (1.000)	0.560 (0.750)	0.800 (0.890)	0.613 (0.772)
Mean	0.362 (0.595)	0.367 (0.600)	0.253 (0.495)	1.027 (1.002)	0.325 (0.565)	0.776 (0.874)	
Interaction (A x B)							
	A		B		AxB		
S.Em. ±	(0.024)		(0.017)		(0.058)		
C.D. at 5%	(0.066)		(0.049)		(0.162)		
Ingredients detail:							
B_1: 100% Bael powder, **B_2:** 96% Bael powder + 3% Black salt + 1% Black pepper, **B_3:** 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper, **B_4:** 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,** **B_5:** 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,** **B_6:** 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.							

(Figures shown in parenthesis are square root transformed value)

4.3.1.4.3. Interaction effect of genotypes and ingredients on titratable acidity

Interaction effect of genotypes and ingredients on titratable acidity of appetizer was found to be significant. Highest value of titratable acidity was noted A_2B_4(Pant Bael-2 with 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper) which was found at par with A_1B_4(Pant Bael-1 with48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). Whereas the minimum value noted in A_1B_1(Pant Bael-1, 100% Bael powder). Similar results also obtained by Abhangrao *et al.* 2017 in Guava R.T.S.

4.3.1.5. Ascorbic acid

The effect of genotypes and ingredients on ascorbic acidof appetizer is presented in Table 4.21 and discussed under following subheading.

4.3.1.5.1 Effect of genotypes on ascorbic acid

The effect of genotype on ascorbic acid content were found to be significant.The highest ascorbic acid contentwas observed inappetizer of Pant Bael-3(16.968mg/100g). Whereas the lowest ascorbic acid content was observed inappetizer of Pant Bael - 15(13.257mg/100g). This variation in ascorbic acid of various genotypes might be due to the difference in their genetic constitution. Similar effect of genotype on ascorbic acid content of value added product was reported by Jain and Nema, (2007), Choudhary *et al.* (2008), Pawar and Patil (2013) and Abhangrao *et al.* (2017).

4.3.1.5.2 Effect of ingredients on ascorbic acid

An effect of ingredients on ascorbic acid were found to be significant.The maximum ascorbic acid content was reported from B_4 (33.308mg/100g) which was significantly superior over other ingredients. It was followed by B_6 (22.904mg/100g). However, the minimum ascorbic acid content was reported from B_3 (5.526mg/100g). This might be due to the fact that, among the different ingredients used for appetizer preparation aonla consist the high ascorbic acid content andmost of its ascorbic acid contentmaintain even after processing. Jain and Nema, (2007) and Abhangrao *et al.* (2017) also reported the similar effect of ingredients on ascorbic acid content of the fruit based value added product.

Table 4.21 : Effect of genotypes and ingredients on ascorbic acid of appetizer

Treatments	Ascorbic acid (mg/100g)						
	Ingredients						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	9.300	9.010	6.200	35.050	12.560	24.380	16.083
A_2 :(Pant Bael-2)	8.401	8.431	5.750	34.200	12.110	23.930	15.470
A_3 :(Pant Bael-3)	10.700	10.520	6.900	35.350	13.260	25.080	16.968
A_4 :(Pant Bael-4)	8.660	8.340	5.880	34.330	12.240	24.060	15.585
A_5 :(Pant Shivani)	7.500	7.330	5.250	33.750	11.660	23.480	14.828
A_6 :(Pant Aparna)	6.480	6.050	4.790	31.240	10.150	21.070	13.297
A_7 :(Pant Bael-10)	7.050	7.120	5.075	33.020	11.450	22.100	14.303
A_8 :(Pant Bael-14)	8.300	8.120	5.700	34.150	12.060	23.010	15.223
A_9 :(Pant Bael-15)	6.600	6.660	4.850	30.120	11.210	20.100	13.257
A_{10} :(Pant Bael-16)	7.890	7.720	5.495	33.945	11.855	23.670	15.096
A_{11} :(Gonda No.2)	6.700	6.200	4.900	31.230	10.230	21.060	13.387
Mean	7.962	7.773	5.526	33.308	11.708	22.904	

Interaction (A x B)			
	A	B	AxB
S.Em. ±	0.177	0.131	0.433
C.D. at 5%	0.495	0.366	1.213

Ingredients detail:

B_1: 100% Bael powder,
B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,
B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,
B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**
B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**
B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

4.3.1.5.3. Interaction effect of genotypes and ingredients on ascorbic acid

The interaction effect of genotypes and ingredients on ascorbic acid of appetizer were found to be significant. The maximum ascorbic acid content was found in A_3B_4 (Pant Bael-3 with 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper) which was found at par withA_1B_4 A_2B_4 (), A_4B_4 and A_8B_4. Whereas, the minimum ascorbic acid content was observed from A_6B_3.Similar findings also reported by Abhangrao *et al.* 2017 in Guava R.T.S

4.3.1.6. Moisture

The effect of genotypes and ingredients on moisture of appetizer was found to be significant (Table 4.22) and discussed as below.

4.3.1.6.1. Effect of genotypes on moisture

The effect of genotype on moisture of appetizer was found to be significant. The minimum moisture content was observed in Pant Aparna (4.330%) which was found at par with Pant Shivani(4.667%).Whereas, the highest moisture content was noted in Pant Bael-15(7.25%). The variation in the moisture content of powder might be due to their differential absorbing capacity of moisture from the atmosphere. Pawar and Patil (2013) and Kumari and Khatkar, (2018) also reported the effect of genotype on moisture content of value added product.

4.3.1.6.2. Effect of ingredients on moisture

An effect ingredients moisture of appetizer was found to be significant. The minimum value ofcontent (5.15%)was observed in B_6(24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper). Whereas, the maximum value ofmoisture content (6.34%) was noted in B_5 (48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper). This might be due the fact that variation in the moisture content of ingredients due to their differential absorbing capacity of moisture from the atmosphere which effects the moisture content of appetizer.Similar effect of ingredients on different value added product was reported by Bhatt and Verma, (2016) in Bael toffee and Jain and Nema, (2007) in guava leather.

Table 4.22: Effect of genotypes and ingredients on moisture of appetizer

Treatments	Moisture(%)						
	Ingredients						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	5.000 (2.447)	5.000 (2.447)	4.400 (2.321)	4.400 (2.321)	6.000 (2.644)	4.600 (2.364)	4.900 (2.424)
A_2 :(Pant Bael-2)	5.400 (2.528)	5.400 (2.528)	6.200 (2.682)	5.000 (2.447)	6.400 (2.719)	6.800 (2.791)	5.867 (2.616)
A_3 :(Pant Bael-3)	7.200 (2.862)	7.200 (2.862)	5.800 (2.606)	6.800 (2.791)	6.200 (2.682)	5.400 (2.528)	6.443 (2.722)
A_4 :(Pant Bael-4)	7.800 (2.965)	7.600 (2.931)	7.800 (2.965)	6.600 (2.755)	7.600 (2.931)	5.800 (2.606)	7.220 (2.859)
A_5 :(Pant Shivani)	6.800 (2.791)	6.800 (2.791)	4.000 (2.233)	4.400 (2.321)	3.200 (2.046)	2.800 (1.945)	4.667 (2.355)
A_6 :(Pant Aparna)	3.600 (2.142)	3.000 (1.996)	3.400 (2.094)	4.400 (2.321)	6.800 (2.791)	4.660 (2.364)	4.330 (2.285)
A_7 :(Pant Bael-10)	6.800 (2.791)	7.000 (2.827)	6.800 (2.791)	6.800 (2.791)	6.200 (2.682)	5.400 (2.528)	6.500 (2.735)
A_8 :(Pant Bael-14)	3.800 (2.188)	4.000 (2.233)	6.000 (2.644)	4.400 (2.321)	5.200 (2.488)	3.000 (1.996)	4.400 (2.312)
A_9 :(Pant Bael-15)	7.600 (2.931)	7.600 (2.931)	7.000 (2.827)	6.800 (2.791)	7.800 (2.965)	6.800 (2.791)	7.267 (2.873)
A_{10} :(Pant Bael-16)	4.800 (2.406)	4.800 (2.406)	5.800 (2.606)	6.400 (2.719)	7.660 (2.931)	6.400 (2.719)	5.967 (2.631)
A_{11} :(Gonda No.2)	4.400 (2.321)	4.400 (2.321)	6.200 (2.682)	6.000 (2.644)	7.000 (2.827)	5.000 (2.447)	5.500 (2.541)
Mean	5.745 (2.580)	5.709 (2.571)	5.764 (2.587)	5.636 (2.566)	6.364 (2.701)	5.145 (2.462)	
Interaction (A x B)							
	A		B		AxB		
S.Em. ±	(0.028)		(0.021)		(0.068)		
C.D. at 5%	(0.078)		(0.058)		(0.192)		

Ingredients detail:

B_1: 100% Bael powder,
B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,
B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,
B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**
B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**
B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

(Figures shown in parenthesis are square root transformed value)

4.3.1.6.3. Interaction effect of genotypes and ingredients on moisture

Interaction effect of genotypes and ingredients on moisture of appetizer was found to be significant. The minimum moisture content(3%) was found in $A_6B_2$and A_8B_6which was found at par with A_6B_1, A_6B_3 and A_8B_1. Whereas, the maximum moisture content (7.8%) found in A_4B_1(Pant Bael-4, 100% Bael powder), A_4B_3(Pant Bael-4, 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper) and A_9B_5(Pant Bael-15, 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper).

4.3.1.7. Non enzymatic browning

Thenon enzymatic browning, is a process that also produces the brown pigmentation in foods, but without the activity of enzymes. The effect of genotypes and ingredients on non enzymatic browning was presented in Table 4.23 and discussed as below.

4.3.1.7.1. Effect of genotypes on non enzymatic browning

The effect of genotypes on non enzymatic browning of appetizer was found to be significant. The minimum non enzymatic browning (0.807) was reported in Pant Bael-10. Whereas, the maximumnon enzymatic browning was (1.530) found in Gonda No.2. This might be due to the browning reaction between reducing sugars and amino groups, degradation of ascorbic acid that present in different genotype due to high temperature during drying process.

4.3.1.7.2. Effect of ingredients on non enzymatic browning

The effect of ingredients on non enzymatic browning of appetizer was foundto be significant. The minimum non enzymatic browning(1.033) was noted fromB_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper). Whereas the, maximum value ofnon enzymatic browning (1.369)was found in B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). The browning reaction between reducing sugars and amino groups, degradation of ascorbic acid that present in different ingredientsdue to high temperature during drying process.

Table 4.23: Effect of genotypes and ingredients on non enzymatic browning of appetizer

Treatments	Non enzymatic browning						
	Ingredients						
Genotypes	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	1.020	1.020	0.920	0.760	1.000	1.010	0.955
A_2 :(Pant Bael-2)	1.500	1.480	0.850	0.700	1.510	1.330	1.228
A_3 :(Pant Bael-3)	1.550	1.560	1.110	1.250	1.510	1.410	1.397
A_4 :(Pant Bael-4)	1.630	1.620	1.210	1.310	1.610	1.420	1.467
A_5 :(Pant Shivani)	0.740	0.630	0.630	1.610	1.060	1.270	0.990
A_6 :(Pant Aparna)	1.660	1.660	1.280	1.680	1.300	1.500	1.513
A_7 :(Pant Bael-10)	0.630	0.710	0.630	1.640	0.870	0.360	0.807
A_8 :(Pant Bael-14)	1.600	1.520	1.190	1.680	1.360	1.100	1.408
A_9 :(Pant Bael-15)	1.410	1.450	1.010	1.340	1.630	1.480	1.387
A_{10} :(Pant Bael-16)	1.680	1.630	1.190	1.340	1.611	1.421	1.479
A_{11} :(Gonda No.2)	1.629	1.628	1.348	1.410	1.600	1.561	1.530
Mean	1.368	1.355	1.033	1.338	1.369	1.260	

Interaction (A x B)			
	A	B	AxB
S.Em. ±	0.014	0.010	0.034
C.D. at 5%	0.039	0.029	0.096

Ingredients detail:

B_1: 100% Bael powder,

B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,

B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,

B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**

B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**

B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

4.3.1.7.3. Interaction effect of genotypes and ingredients on non enzymatic browning

An significant interaction effect of genotypes and ingredients on non enzymatic browning of appetizer was observed.The minimum valuenon enzymatic browning (0.360) was found inA_7B_6 (Pant Bael-10 with 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper). Whereas, the maximum value non enzymatic browning was noted in (1.680) inA_6B_4 (Pant Aparna, 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper),A_8B_4(Pant Bael-10, 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper) and $A_{10}B_1$ (Pant Bael-16, 100% Bael powder)

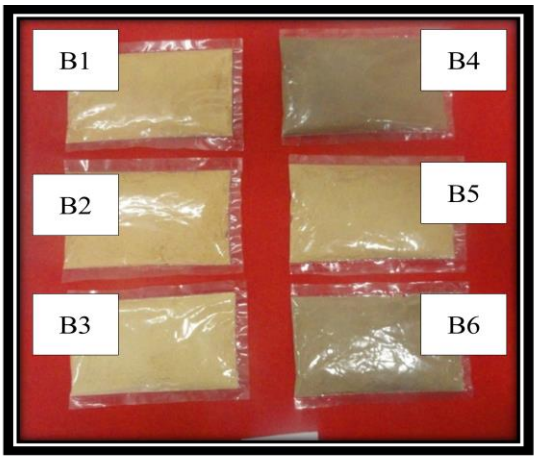
4.3.2. Sensory evaluation

The sensory evaluation or sensory analysis is a scientific discipline that applies the principles of experimental designs and statistical analysis to the use of human senses(sight, smell, taste, touch and hearing) for the purpose of evaluating consumer products.It requires the panels of human assessors, those who tested the product and recording the responses made by them. Then by applying statistical techniques to the results obtain from human assessors it will be possible to make an insights and inferencesabout the products that was under test. It is generally the final guide of the product quality from the consumer's point of view and it is an important parameter in determining the quality. It revolves around the colour, flavour, taste, texture, and overall acceptability of product. Sensory evaluation of appetizer describes in detail under following subheading.

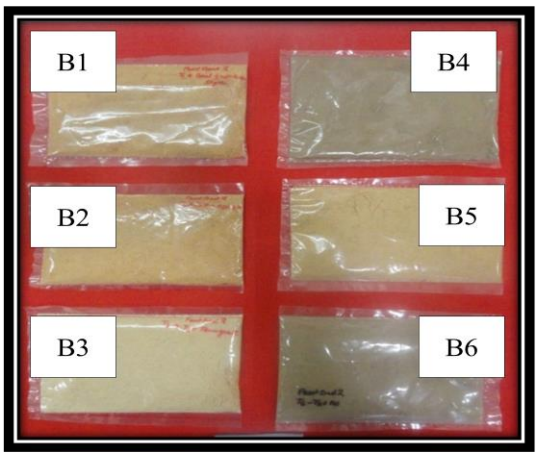
4.3.2.1.Colour

The colour attracts first the consumer toward the product and can help in impulse purchases. At the point of purchase consumers uses mostly appearance factors to provide an indication of quality. Colour is derived from the natural pigments present in fruits. The primary pigments which imparting colour quality are the fat soluble chlorophylls (green) and carotenoids (orange, yellow and red) and the water soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red). In addition to these, the enzymatic and non-enzymatic browning reactions may result in the formation of water soluble brown, gray, and black coloured pigments.

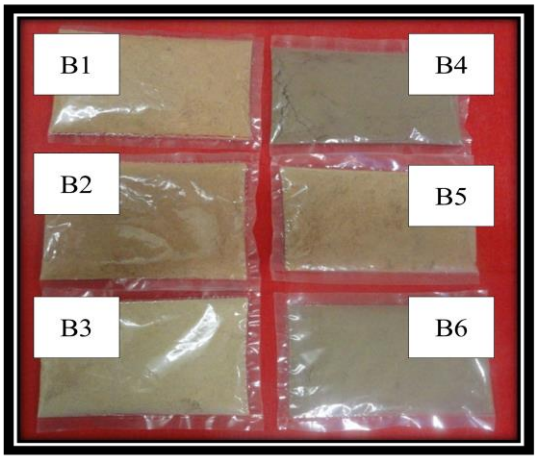
Plate 2: Appetizer developed from different genotypes and ingredients.



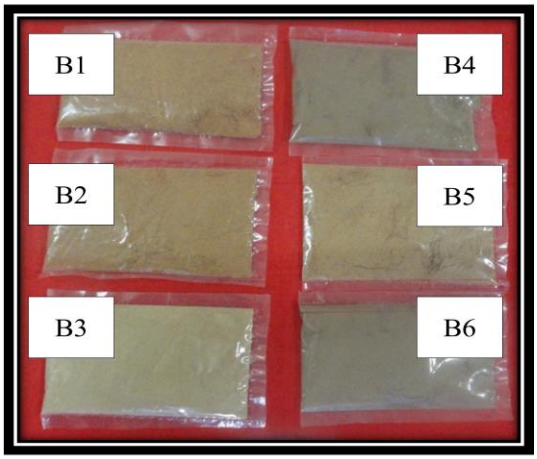
Pant Bael -1



Pant Bael -2

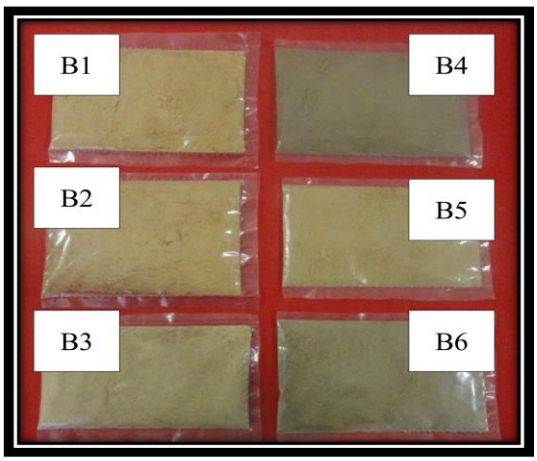


Pant Bael -3

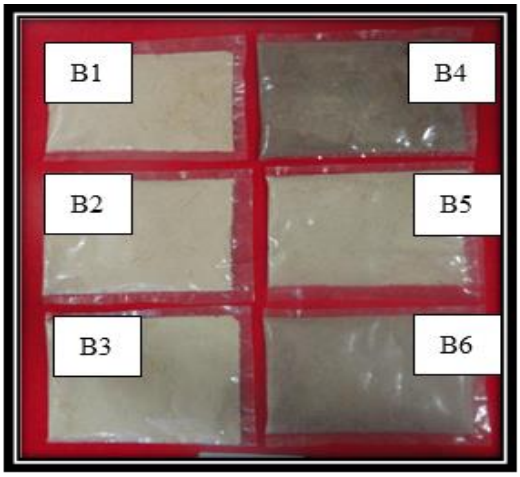


Pant Bael -4

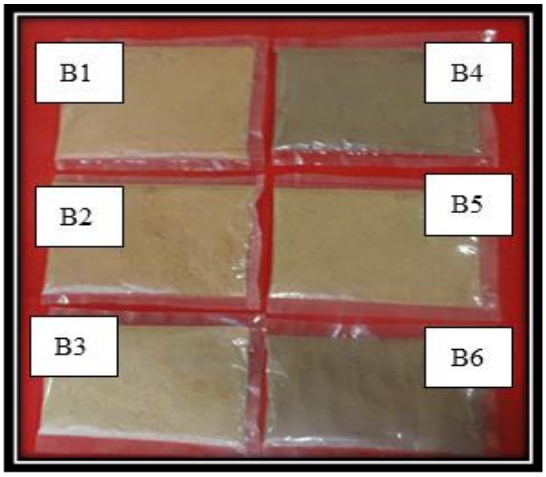
Pant Shivani



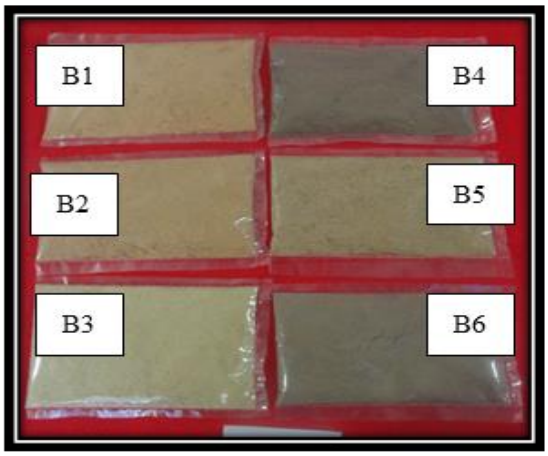
Pant Aparna



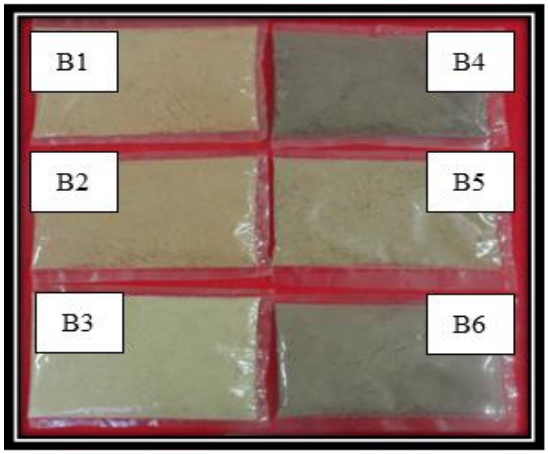
Pant Bael 10



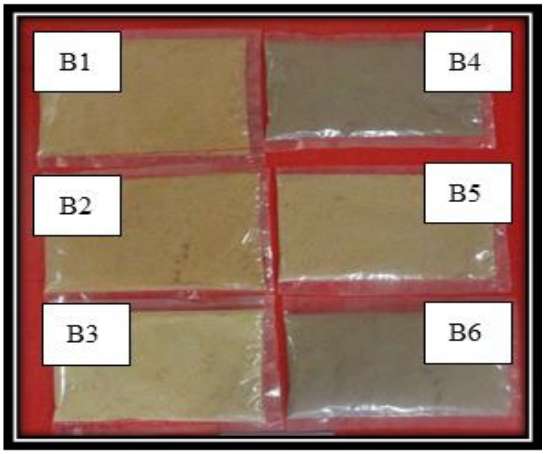
Pant Bael 14



Pant Bael 15



Pant Bael 16



Gonda no. 2

An effect of genotypes and ingredients on colour of appetizer is presented on Table 4.24 and describe in detail as below.

4.3.2.1.1. Effect of genotypes on colour

The effect of genotype on colour of appetizer was found to be non significant. The maximum score was 8.1 (Like very much) and the minimum score is 7.0 (Like moderately). The genotype Pant Bael-1, Pant Shivani, Pant Aparna, Pant Bael-10,Pant bael-14 obtained maximum score 8 (Like very much). Whereas, the minimum score 7 (Like moderately) obtainedin Pant Bael-2, Pant Bael-3, Pant Bae-l4, Pant Bael-15, Pant Bael-16 and Gonda No.2.

4.3.2.1.2. Effect of ingredients on colour

An effect of ingredients on colour of appetizers was found to be significant. The maximum score was 7.9 (Like very much) and the minimum score is 7.01 (Like moderately). The maximum score was found in B_5 (48% Bael powder + 48% Jamun seed p owder + 3% Black salt + 1%Black pepper) which was found significantly superior over other ingredients. Whereas, the minimum score was obtained from the rest of ingredients.Different ingredients used in preparation of appetizer. Each ingredient has its own unique colour, which also pose the significant effect on colour of appetizer. An effect of ingredients on colour of product is also reported by, Elbelazi *et al.* (2015) highest ranked for colour were observed when 1.5g spices + 2g citric acid per kg apple pulp was used for value added apple butter preparation.Similar effect of ingredients on colour of value added product of Bael was also reported by Kaur and Kochhar (2017),Thukral (2017) and Ullikashi *et al.* 2017.The best product with respect to colour was obtained when50% level of aonla pulp and 50% of bael pulp used for preparation of mixed fruit leather by Uttarwar *et al.* 2018.

4.3.2.1.3. Interaction effect of genotypes and ingredients on colour

The interaction effect of of genotypes and ingredients on colour of appetizer is found to be non significant. However, the maximum score obtained was 9(Like extremely) and the minimum score was 6(Like slightly).

Table 4.24 : Effect of genotypes and ingredients on colour of appetizer

Treatments	Colour						
Genotypes	**Ingredients**						
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	7.666	7.666	7.833	7.333	8.000	6.833	7.555
A_2 :(Pant Bael-2)	6.333	6.333	8.000	5.333	8.666	7.333	7.000
A_3 :(Pant Bael-3)	7.666	6.333	6.666	7.333	7.333	7.000	7.055
A_4 :(Pant Bael-4)	6.500	7.333	6.000	6.333	8.333	7.333	6.972
A_5 :(Pant Shivani)	7.333	8.000	8.000	7.666	7.333	8.000	7.722
A6 :(Pant Aparna)	8.500	8.000	7.833	8.166	8.666	7.833	8.166
A_7 :(Pant Bael-10)	7.333	7.333	9.000	7.666	8.166	7.166	7.777
A_8 :(Pant Bael-14)	6.500	7.500	8.666	7.333	7.666	7.666	7.555
A_9 :(Pant Bael-15)	7.333	7.333	5.666	5.666	8.666	8.333	7.166
A_{10} :(Pant Bael-16)	8.000	7.333	8.333	7.000	7.000	6.000	7.277
A_{11} :(Gonda No.2)	7.000	6.666	7.000	7.333	7.333	7.000	7.055
Mean	7.288	7.255	7.544	7.011	7.922	7.311	

Interaction (A x B)			
	A	B	AxB
S.Em. ±	0.042	0.023	0.2507
C.D. at 5%	NS	0.361	NS

Ingredients detail:

B_1: 100% Bael powder,

B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,

B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,

B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper**,**

B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**

B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper.

4.3.2.2. Flavour

Flavour is a mingled but an unitary experience which includes sensations of taste, smell, and pressure. Flavor is typically described by aroma and taste. The perusal of data on Table 4.25 shows the effect of genotypes and ingredients on flavour of appetizer which is describe as below.

4.3.2.2.1. Effect of genotypes on flavour

The effect of genotypes on flavour of appetizer was found to be significant. The maximum score 8.1 (Like very much) was obtained in Pant Aparna followed by Pant Bael-1 and Pant Bael-10.Whereas, the minimum score 6.5 (Like moderately) was observed in Pant Bael-2and Pant Bael-3. The Bael fruit are highly aromatic and uniqueness of genotypes added flavour to the appetizer. Similar effect of fruit genotype on flavour of value added product Sanford *et al.* (2001), Jain and Neema (2007) and Zeenath *et al.* (2016).

4.3.2.2.2. Effect of ingredients on flavour

The effect of ingredients on flavour of appetizer was found to be significant. The maximum score 7.9 (Like very much) was noted in B_5 (48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper) which was found significantly superior over other ingredients. However, the minimum score 7.2 (Like moderately) noted in B_2 (96% Bael powder + 3% Black salt + 1% Black pepper). The maximum score obtained in B_5 might be due to the blend of Bael powder and jamun seed powder resulted in good flavour. These effects was due to the unique flavour of each ingredient used for appetizer preparation. In the year 2017, similar finding on effect of ingredients on flavour of Bael based value added product was reported byKaur and Kochhar (2017), Thukral (2017) and Ullikashi *et al.* (2017). Similar effect was also reported by Uttarwar *et al.* (2018) in preparation of mixed fruit leather and the highest score obtained from 50% level of aonla pulp and 50% of Bael pulp with respect to flavour.

4.3.2.2.3. Interaction effect of genotypes and ingredients on flavour

An interaction effect of genotypes and ingredients on flavour was found to be non significant. However, the maximum score obtained was 9.0 (Like extremely) and the minimum score was 5.3 (Neither like nor dislike). The highest score noted from A_7B_3 (Pant Bael-10 with 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper) and lowest score from A_2B_1(100% Bael powder of Pant Bael-2).

Table 4.25: Effect of genotypes and ingredients on flavour of appetizer

Treatments	Flavour						
Genotypes	**Ingredients**						
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	7.333	8.000	7.333	8.000	8.000	8.000	7.777
A_2 :(Pant Bael-2)	5.333	6.000	7.333	5.666	8.000	6.666	6.500
A_3 :(Pant Bael-3)	7.666	6.333	6.666	6.666	7.666	7.166	7.027
A_4 :(Pant Bael-4)	6.833	7.000	6.666	8.166	8.000	8.333	7.500
A_5 :(Pant Shivani)	7.333	7.333	7.333	8.000	7.333	8.333	7.611
A_6 :(Pant Aparna)	8.666	7.833	7.833	8.000	8.333	8.000	8.111
A_7 :(Pant Bael-10)	7.666	7.333	9.000	8.333	8.166	7.733	8.038
A_8 :(Pant Bael-14)	8.000	7.833	7.500	7.333	7.833	7.333	7.638
A_9 :(Pant Bael-15)	8.000	7.333	7.000	7.000	8.000	8.000	7.555
A_{10} :(Pant Bael-16)	8.000	7.333	8.333	7.000	8.000	7.000	7.611
A_{11} :(Gonda No.2)	8.666	7.333	7.000	7.666	7.666	7.333	7.611
Mean	7.599	7.244	7.455	7.433	7.900	7.622	

Interaction (A x B)			
	A	B	AxB
S.Em. ±	0.029	0.016	0.173
C.D. at 5%	0.338	0.247	NS

Ingredients detail:

B_1: 100% Bael powder,

B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,

B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,

B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper**,**

B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**

B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper

4.3.2.3. Taste

The sensation that perceived in the mouth and throat on contact with a substance is called as taste. It includes the sweet, sour, salty and bitter quality of a thing that can sense when it is in the mouth. Taste is important for the further acceptability of any product. The data pertaining effect of genotypes and ingredients on taste of appetizers presented on Table 4.26 and describe under following subheading.

4.3.2.3.1. Effect of genotypes on taste

The effect of genotypes on taste of appetizers was found to be significant. The maximum score 8.16 (Like very much) was obtained in Pant Bael-10, which was found at par with Pant Aparna, Pant Bael-1,Pant Bael-14 and Pant Bael-15. Whereas, minimum score 6.22 (Like slightly) was noted from Pant Bael-2. The effect on taste is due to uniqueness of genotypes. Similar effect of genotype on taste on value added product was observed by Jain and Neema (2007) in guava andZeenath *et al.,* (2016) in mango.

4.3.2.3.2. Effect of ingredients on taste

The effect of ingredients on taste of appetizers was found to be significant. The maximum score 7.7 (Like very much) obtained in B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper) followed by B_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper) and B_6 (24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper). The minimum score of 7(Like moderately) noted in B_1(100%Bael powder).The distinctive taste of each ingredient adds unique taste to the appetizer. The similar effect of ingredients on the Bael based value added product was reported by **Liyanaduragc *et al*.(2007),** Kaur and Kochhar (2017), Thukral (2017) and Ullikashi *et al.* (2017) andUttarwar *et al.* (2018).

4.3.2.3.3. Interaction effect of genotypes and ingredients on taste

An interaction effect of genotypes and ingredients on taste of appetizer was found to be non significant. However, the maximum score is 9 (Like extremely)obtained in A_4B_6 (Pant Bael-4 with 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper) and A_7B_3 (Pant Bael-10 with 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper. Whereas, the minimum score of 5(Neither like nor dislike) noted from A_2B_1(100% Bael powder of genotype Pant Bael -2).

Table 4.26 : Effect of genotypes and ingredients on taste of appetizers

Treatments	Taste						
Genotypes	**Ingredients**						
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	7.833	8.000	8.166	7.500	7.833	7.833	7.861
A_2 :(Pant Bael-2)	4.666	5.333	7.000	5.666	8.000	6.666	6.222
A_3 :(Pant Bael-3)	7.666	6.333	7.333	8.000	6.333	6.000	6.944
A_4 :(Pant Bael-4)	6.666	7.666	7.000	7.333	8.333	9.000	7.666
A_5 :(Pant Shivani)	6.666	7.333	7.333	8.000	7.333	8.333	7.500
A_6 :(Pant Aparna)	8.666	8.166	8.000	8.000	8.000	8.000	8.138
A_7 :(Pant Bael-10)	8.166	7.333	9.000	8.333	8.166	8.000	8.166
A_8 :(Pant Bael-14)	8.166	8.000	7.000	8.333	7.333	8.333	7.861
A_9 :(Pant Bael-15)	7.333	8.333	7.333	8.000	7.666	8.000	7.777
A_{10} :(Pant Bael-16)	7.833	8.333	8.166	8.000	6.333	7.000	7.611
A_{11} :(Gonda No.2)	7.500	6.500	6.500	7.666	6.000	7.500	6.944
Mean	7.377	7.399	7.533	7.711	7.399	7.699	
Interaction (A x B)							
	A		B		AxB		
S.Em. ±	0.036		0.020		0.219		
C.D. at 5%	0.424		0.314		NS		
Ingredients detail:							
B_1: 100% Bael powder, **B_2:** 96% Bael powder + 3% Black salt + 1% Black pepper, **B_3:** 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper, **B_4:** 48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper**,** **B_5:** 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,** **B_6:** 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper							

4.3.10. Texture

Texture is another important sensoary parameter to judge the quality of product. The textural parameters are perceived with the sense of touch or either when the product is picked up by hand or placed in the mouth and chewed. The data in respect of texture of appetizers as influenced by genotypes and ingredients presented in Table 4.27 and discussed as below.

4.3.10.1. Effect of genotypes on texture

The effect of genotype on texture was found to be significant.The maximum score 8.138(Like very much) was noted in Pant Aparna which was found at par with Pant Bael-10,Pant Bael-1 andPant Bael-16.Where as the minimum score 6.223 and 6.472 which mean (Like slightly) noted in Pant Bael-2 and Pant Bael-3. Jain and Neema (2007) and Zeenath *et al.* (2016) also reported the same effect on texture.

4.3.10.2. Effect of ingredients on texture

The effect of ingredients on texture was found to be significant. the maximum score of 7.711(Like very much) wasfound in B_6 (24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper), which was found at par with B_2 (96% Bael powder + 3% Black salt + 1% Black pepper), B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper) and B_5 (48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper). Whereas minimum score 7.044 and 7. 066 obtained which mean Like moderately from B_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper) and B1 (100% Bael powder). Similar effect on texture of different Bael based value added product was found by Liyanaduragc *et al*. (2007), Pingale and Dighe (2015), Bhatt and Verma (2016), Kaur and Kochhar (2017), Thukral (2017) and Ullikashi *et al.* (2017) andUttarwar *et al.* (2018).

4.3.10.3. Interaction effect of genotypes and ingredients on texture

An significant interaction effect of genotypes and ingredients on texture of appetizer was observed presented in table. The maximum score 8.666(Like extremely) was obtained from A_4B_6 (Pant Bael-4 with 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper)

Table 4.27: Effect of genotypes and ingredients on texture of appetizers

Treatments	Texture						
Genotypes	**Ingredients**						
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	7.166	8.000	7.833	7.333	8.000	7.666	7.666
A_2 :(Pant Bael-2)	5.666	6.000	6.000	5.666	7.000	7.000	6.223
A_3 :(Pant Bael-3)	4.666	6.333	6.666	7.000	7.333	6.833	6.472
A_4 :(Pant Bael-4)	7.000	7.500	6.166	7.166	8.333	8.666	7.472
A_5 :(Pant Shivani)	7.000	7.333	7.333	8.000	7.333	8.333	7.555
A_6 :(Pant Aparna)	8.500	8.000	7.833	8.333	8.166	8.000	8.138
A_7 :(Pant Bael-10)	8.000	7.333	8.666	8.000	8.000	8.000	8.000
A_8 :(Pant Bael-14)	8.000	8.000	6.666	7.666	7.333	7.666	7.555
A_9 :(Pant Bael-15)	7.333	7.666	6.666	7.666	6.666	7.333	7.222
A_{10} :(Pant Bael-16)	7.666	8.000	7.000	8.000	7.333	8.000	7.666
A_{11} :(Gonda No.2)	6.666	7.000	6.666	7.666	7.000	7.333	7.055
Mean	7.066	7.377	7.044	7.500	7.500	7.711	
Interaction (A x B)							
	A		B		AxB		
S.Em. ±	0.042		0.229		0.252		
C.D. at 5%	0.493		0.366		1.218		

Ingredients detail:

B_1: 100% Bael powder,

B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,

B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,

B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**

B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**

B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper

followed by A_7B_3 (Pant Bael-10 with48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper)and A_6B_1 (Pant Aparna with 100% Bael powder). However the minimum score of 4.666(Neither like nor dislike) was obtained from A_2B_1 (Pant Bael- 3, 100% Bael powder). Similar effect of genotypes and ingredients on texture of value added product was reported by Neema and Jain(2007).

4.3.11. Overall acceptability

The colour and appearances decide the first purchase of the product but ultimatelythe overall acceptability of the product is the most important for its further future purchase. The data in respect to overall acceptability as influenced by genotypes and ingredients is presented in Table 4.28.

4.3.11.1. Effect of genotypes on overall acceptability

The effect of genotypes on overall acceptability of appetizer is found to be significant. The maximum score8.115 (Like very much) was obtainedin Pant Bael-15, which was found at par withPant Bael-10, Pant Bael-14 and Pant Bael-16.whereas the minimum score 7.055(Like moderately) noted in Pant Bael-3 and Gonda No.2. The uniqueness of each genotype affects the overall acceptability of the appetizer. Similar effect on overall acceptability of different fruit variety based value added product by Jain and Nema(2007), Zeenath *et al.* (2016) and Mishra and Krska(2017)

4.3.11.2. Effect of ingredients on overall acceptability

The effect of ingredients on overall acceptability of appetizer was found to be significant. The maximum score 7.883(Like very much) was observed in B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Blacksalt + 1% Black pepper) and B_5 (48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper). Whereas, the minimum score 7.444noted inB_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper). Among the different ingredients used for mouth freshener preparation, 50% dehydrated aonla pulp, 15% fennel, 10% arecanut and 20% sugar was adjudged best on the basis of overall sensory acceptability attributes (Barwal *et.al.* 2010). A Similar effect on overall acceptability of different Bael based value added product was found by Liyanaduragc *et al*. (2007), Pingale and Dighe (2015), Bhatt and Verma (2016), Kaur and Kochhar (2017), Thukral (2017) and Ullikashi *et al.* (2017) andUttarwar *et al.* (2018).

Table 4.28: Effect of genotypes and ingredients on overall acceptability of appetizers

Treatments	Overall acceptability						
Genotypes	**Ingredients**						
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean**
A_1:(Pant Bael-1)	7.500	7.666	7.833	7.333	7.666	7.333	7.555
A_2 :(Pant Bael-2)	6.666	7.666	7.333	8.000	8.000	6.333	7.333
A_3 :(Pant Bael-3)	7.666	6.666	6.666	7.000	7.500	6.833	7.055
A_4 :(Pant Bael-4)	6.666	8.166	6.166	7.500	8.333	8.500	7.555
A_5 :(Pant Shivani)	7.666	8.233	7.900	8.000	5.333	6.000	7.188
A6 :(Pant Aparna)	8.000	6.666	7.666	7.666	8.000	7.666	7.619
A_7 :(Pant Bael-10)	7.833	7.333	8.833	8.166	8.166	8.166	8.083
A_8 :(Pant Bael-14)	7.666	7.833	7.333	8.166	7.666	8.333	7.833
A_9 :(Pant Bael-15)	7.833	8.333	7.333	8.200	8.666	8.333	8.116
A_{10} :(Pant Bael-16)	8.000	8.333	8.166	8.500	7.333	8.000	8.055
A_{11} :(Gonda No.2)	6.666	7.000	6.666	7.666	7.000	7.333	7.055
Mean	7.466	7.619	7.444	7.833	7.600	7.533	

Interaction (A x B)

	A	B	AxB
S.Em. ±	0.034	0.018	0.203
C.D. at 5%	0.407	0.299	NS

Ingredients detail:

B_1: 100% Bael powder,

B_2: 96% Bael powder + 3% Black salt + 1% Black pepper,

B_3: 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper,

B_4: 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper**,**

B_5: 48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1%Black pepper**,**

B_6: 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper

4.3.11.3. Interaction effect of genotypes and ingredients on overall acceptability

An effect of interaction of genotypes and ingredients on overall acceptability was found to be non significant. However the maximum score obtained was 8.833(Like extremely) observed in A_7B_3(Pant Bael-10 with followed by 48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper). Whereas, theand minimum score obtained was 6(Like slightly) from A_5B_6(Pant Shivani with 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper).

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLSION

The investigation entitled Biochemical and molecular characterization of diverse genotypes of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties was conducted at the Department of Horticulture, Patharchatta, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, district Udham Singh Nagar (Uttarakhand) during 20016-18. The experiment was conducted in three parts. In the first part an investigation on biochemical parameters was laid out in Completely randomized design (CRD) with 18 Treatment (genotypes) and three replication. In the second part microsatellite (SSR) study conducted in 24 genotype of Bael. The third part consist of preparation of nutraceutically rich appetizer from eleven different genotypes of Bael, six ingredients with sixty six treatment combination and experiment was laid out in FCRD (Factorial Completely Block Design). The experimental findings obtained during course of investigation are summarized below:

1. Analysis of variance revealed significant difference among the Bael genotypes for all the biochemical parameters.

2. The maximum ascorbic acid content was observed from Pant Bael-3 and Pant Bael-1. However, the minimum ascorbic acid content was obtained from Haldi Nurmohamd.

3. The maximum value of marmelosin was found in Pant Bael -1, Pant Bael -15 and Pant Bael- 13. Whereas, the minimum value of marmelosin is obtained from Haldi Nurmohamd.

4. The highest total phenol content in noted from Pant Sujata and the lowest total phenol content was obtained from the Pant Bael-16. The maximum pectin content was obtained from Haldi Nurmohamd, Pant Bael- 3 Pant Bael-16. The minimum pectin content was obtained from Pant Bael -2.

5. The highest riboflavin was found in Pant Sujata, Pant Aparna and Pant Shivani. The lowest riboflavin was noted in Haldi Nurmohamd-2.

6. The maximum total carotenoids were noted from Pant Bael -1 and Pant Bael-2. Whereas, the minimum total carotenoids was noted in Pant Bael-10. The

highest total flavonoids was observed in Pant Aparna followed by Pant Sujata and Pant Shivani. However, the lowest total flavonoids was found in Patharchatta -1.

7. The maximum enzymatic antioxidant activity of SOD and CAT was found in Pant Shivani. The maximum POD enzyme activity was reported from Pant Bael-1. The maximum DPPH radical scavenging activity reported from Pant Shivani and minimum DPPH radical scavenging activity observed in Pant Bael-1.

8. The maximum TSS was obtained from Pant Bael -15 and, the minimum TSS was obtained from Faizabad No.9.

9. The maximum phenylalanine ammonia lyase enzyme acitivity was noted from Pant Aparna, Pant Sujata and Pant Shivani. Whereas, minimum phenylalanine ammonia lyase enzyme acitivity reported from Patharchatta-1. The minimum value of polyphenol oxidase enzyme activity noted in Haldi Nurmohamd, the maximum value of polyphenol oxidase enzyme activity is found in Pant Bael -4.

10. The biochemical analysis revealed that Pant Bael-1, Pant Sujata, Pant Bael-3, Pant Shivani and Pant Aparna were found suitable for future breeding programme as they had more than one desirable biochemical character.

11. The genetic variation at DNA level was found to be polymorphic for Bael.

12. A total of 59 SSR loci from 31 primers were detected from 24 genotype of Bael. The level of polymorphism was 100 %. The PCI content was ranged from. The amplified product size range of 100-800bp for Bael genotype.

13. The Jaccard's similarity matrix indicates that similiarity coefficient for Bael genotypes were ranged from 0.251 to 0.951.

14. The UPGMA cluster analysis cataogeries 24 genotypes of Bael into two major clusters.

15. The quality analysis of appetizer revealed that significant effect of genotypes, ingredients and their interaction on different quality parameters *i.e.,* total sugar, reducing sugar, non reducing sugar, titratable acidity, moisture, non enzymatic browning and ascorbic acid content.

16. Among the different genotype used in preparation of appetizer maximum total sugar, reducing sugar and non reducing sugar was obtained from Pant Bael-4. The highest titratable acidity and ascorbic acid and was found in Pant Bael-3. The minimum moisture and non enzymatic browning was obtained from Pant Aparna and Pant Bael-10 repectively.

17. Among the different ingredients used maximum total sugar, reducing sugar, titratable acidity, ascorbic acid content was found in B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). The minimum moisture and non enzymatic browning was obtained from B_6 (24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper) and B_3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper) respectively.

18. Among the different treatment combination maximum total sugar, reducing sugar, non reducing sugar and ascorbic acid content A_8B_4 (Pant Bael-14 with 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper) and maximum titratable acidity obtained from A_2B_4 (Pant Bael-2 with 48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). The minimum moisture and non enzymatic browning was obtained from A_8B_6 (Pant Bael-14 with with 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper) and A_7B_6 (Pant Bael-10 with 24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper) respectively.

19. The sensory evaluation of appetizer revealed the significant effect of genotypes, ingredients and their interaction on different sensory parameters *i.e.,* colour, flavour, taste, texture and over all acceptability. The highest score obtained for different sensory parameters was 9 (Like extremely) and the lowest score was 5 (Neither like or nor dislike).

20. Among the different genotypes maximum score for colour and flavour observed in Pant Bael-1, Pant Aparna and Pant Bael-10. Highest score for taste was obtained from Pant Aparna and Pant Bael-10. The maximum score

for texture obtained in Pant Bael-1 and Pant Aparna. For the overall acceptability maximum score found in Pant Bael-15, Pant Bael-10, Pant Bael-14 and Pant Bael-16.

21. Among the different ingredients maximum score for colour and flavour was noted in B_5. The maximum score for taste and texture was found in B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper). The highest score for overall acceptability was obtained form B_4 (48% Bael powder + 48% Aonla fruit powder + 3% Black salt + 1% Black pepper) and B_5 (48% Bael powder + 48% Jamun seed powder + 3% Black salt + 1% Black pepper). In the interaction effect maximum score for color, flavour, taste, texture and overall acceptability was obtained from A_7B_3 (Pant Bael-10 with followed by 48% Bael powder + 48% Fenugreek seed powder + 3% Black salt + 1% Black pepper).

Conclusion:

From the above study it was concluded that the significant variation exist among the diverse genotypes of Bael based on biochemical and molecular characterization. The fruits of different Bael genotypes found rich in antioxidant properties. The Bael genotypes Pant Bael-1, Pant Sujata, Pant Bael-3, Pant Shivani and Pant Aparna exhibits more than one desirable biochemical characters. The significant variation exist among the genotypes based on biochemical characters but with the use of SSR markers, assessment of the genetic diversity can also help us to plan a future breeding programme using the diverse parent. Besides this, the different Bael genotypes exhibit the promising potential for development of nutraceutically rich appetizer.

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Appendices

APPENDIX I

Weekly weather data during the crop season of Bael in 2016-17

Week no. and Month	Date with duration	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	Evaporation
		Max.	Min.	Max.	Min.		
Jun	05-11	35.3	26.0	68.3	53.7	2.5	6.7
Jun	12-18	35.1	26.1	79.4	55.3	5.8	6.2
Jun	19-25	32.9	25.5	86.3	68.4	15.1	4.7
June-July	26-2	35.9	25.6	76.3	54.0	0.2	6.3
July	3-9	31.0	25.8	94.0	83.0	206.8	4.8
July	10-16	31.7	25.9	90.0	83.0	72.0	4.1
July	17-23	33.9	26.2	87.0	78.0	80.4	4.7
July	24-30	33.2	25.2	88.0	70.0	42.8	4.3
July-Aug	31-6	31.2	25.7	91.0	82.0	203.8	3.7
Aug	7-13	31.8	26.0	91.0	77.0	147.8	4.7
Aug	14-20	32.7	25.6	92.0	71.0	118.8	4.2
Aug	21-27	31.8	25.6	88.0	77.0	57.6	3.7
Aug-Sep	28-3	31.8	25.0	89.0	76.0	129.0	3.9
Sep	4-10	32.0	24.0	90.0	66.0	56.0	4.0
Sep	11-17	34.0	25.2	86.0	64.0	0.0	4.2
Sep	18-25	32.6	24.6	92.0	74.0	168.0	3.2
Sep-Oct	26-2	32.3	23.4	88.0	65.0	7.2	3.2
Oct	3-9	33.1	23.8	88.0	61.0	0.0	2.6
Oct	10-16	33.6	20.3	78.0	48.0	0.0	3.2
Oct	17-23	33.5	18.0	86.0	50.0	0.0	3.1
Oct	24-30	30.8	14.5	87.0	46.0	0.0	2.7
Oct-Nov	31-6	28.4	14.7	90.0	53.0	0.0	2.3
Nov	7-13	28.4	14.7	90.0	53.0	0.0	2.3
Nov	14-20	28.7	12.8	94.0	52.0	0.0	2.0
Nov	21-27	27.9	11.3	86.0	40.0	0.0	2.0
Nov-Dec	28-4	26.1	8.6	93.0	43.0	0.0	2.5
Dec	5-11	24.7	7.8	92.0	48.0	0.0	1.9
Dec	12-18	23.3	10.9	93.0	60.0	0.0	1.6
Dec	19-25	23.1	11.4	94.0	66.0	2.8	1.6
Dec-Jan	26-1	21.3	8.3	95.0	64.0	0.0	1.4
Jan	2-8	22.5	7.2	96.0	66.0	0.0	1.0
Jan	9-15	15.2	6.0	94.9	81.4	3.0	0.8
Jan	16-22	12.9	5.3	95.1	79.0	2.6	0.8
Jan	23-29	20.2	6.9	93.3	65.1	2.7	1.2
Jan-Feb	30-6	18.6	6.4	93.6	70.0	4.2	1.1
Feb	12-18	25.2	9.3	90.6	48.7	0.0	1.6
Feb	19-25	26.9	10.4	91.6	44.6	0.0	2.8
Feb-Mar	26-04	27.2	9.2	87.7	39.6	0.1	2.8
Mar	05-11	25.9	10.0	86.1	50.0	0.4	3.3
Mar	12-18	25.6	7.9	88.0	35.6	0.0	3.6
Mar	19-25	30.5	13.9	82.3	38.3	0.0	3.6

APPENDIX II

Weekly weather data during the crop season of Bael in 2017-18

Week no. and Month	Date with duration	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	Evaporation
		Max.	Min.	Max.	Min.		
Jun	05-11	34.8	25.5	68.9	54.3	2.2	6.0
Jun	12-18	34.6	25.6	80.0	55.9	5.5	5.5
Jun	19-25	32.4	25.0	86.9	69.0	14.8	4.0
June-July	26-2	35.4	25.1	76.9	54.6	0.0	5.6
July	3-9	30.5	25.3	94.6	83.6	206.5	4.1
July	10-16	31.2	25.4	90.6	83.6	71.7	3.4
July	17-23	33.4	25.7	87.6	78.6	80.1	4.0
July	24-30	33.7	24.7	88.6	70.6	42.5	3.6
July-Aug	31-6	31.7	25.2	91.6	82.6	203.5	3.0
Aug	7-13	32.3	25.5	91.6	77.6	147.5	4.0
Aug	14-20	33.2	25.1	92.6	71.6	118.5	3.5
Aug	21-27	32.3	25.1	88.6	77.6	57.3	3.0
Aug-Sep	28-3	32.3	24.5	89.6	76.6	128.7	3.2
Sep	4-10	32.5	23.5	90.6	66.6	55.7	3.3
Sep	11-17	34.5	24.7	86.6	64.6	0.0	3.5
Sep	18-25	33.1	24.1	92.6	74.6	167.4	2.5
Sep-Oct	26-2	32.8	22.9	88.6	65.6	6.6	2.5
Oct	3-9	33.6	23.3	88.6	61.6	0.0	1.9
Oct	10-16	33.1	19.8	78.6	48.6	0.0	2.5
Oct	17-23	33.0	17.5	86.6	50.6	0.0	2.4
Oct	24-30	30.3	14.0	87.6	46.6	0.0	2.0
Oct-Nov	31-6	27.9	14.2	90.6	53.6	0.0	1.6
Nov	7-13	27.9	14.2	90.6	53.6	0.0	1.6
Nov	14-20	28.2	12.3	94.6	52.6	0.0	1.3
Nov	21-27	27.4	10.8	86.6	40.6	0.0	1.3
Nov-Dec	28-4	25.6	8.1	93.6	43.6	0.0	1.8
Dec	5-11	24.2	7.3	92.6	48.6	0.0	1.2
Dec	12-18	22.8	10.4	93.6	60.6	0.0	0.9
Dec	19-25	22.6	10.9	94.6	66.6	2.0	0.9
Dec-Jan	26-1	20.8	7.8	95.6	64.6	0.0	0.7
Jan	2-8	22.0	6.7	96.6	66.6	0.0	0.3
Jan	9-15	14.7	5.5	95.5	82.0	3.5	0.1
Jan	16-22	12.4	4.8	95.7	79.6	2.6	0.1
Jan	23-29	19.7	6.4	93.9	65.7	2.7	0.5
Jan-Feb	30-6	18.1	5.9	94.2	70.6	4.2	0.4
Feb	12-18	24.7	8.8	91.2	49.3	0.0	0.9
Feb	19-25	26.4	9.9	92.2	45.2	0.0	2.1
Feb-Mar	26-04	26.7	8.7	88.3	40.2	0.1	2.1
Mar	05-11	25.4	9.5	86.7	50.6	0.4	2.6
Mar	12-18	25.1	7.4	88.6	36.2	0.0	2.9
Mar	19-25	30.0	13.4	82.9	38.9	0.0	2.9

APPENDIX III

ANOVA for the biochemical characterization of Bael genotypes (Experiment 1)

S.NO.	Parameters	Source	2016-17	
			d.f.	S.S.
1.	Ascorbic acid	Treatments	17	420.37
		Error	36	32.67
		Total	53	453.04
2.	Marmelosin	Treatments	17	5.73
		Error	36	0.35
		Total	53	6.07
3.	Total Phenol content	Treatments	17	9357.73
		Error	36	36.00
		Total	53	9393.73
4.	Pectin content	Treatments	17	25.14
		Error	36	3.34
		Total	53	28.48
5.	Riboflavin	Treatments	17	4.25
		Error	36	0.61
		Total	53	4.86
6.	Total carotenoids	Treatments	17	56.18
		Error	36	12.53
		Total	53	68.71
7.	Total Falvonoids	Treatments	17	4797.60
		Error	36	43.56
		Total	53	4841.16
8.	Superoxidase dismutase	Treatments	17	2256.32
		Error	36	0.01
		Total	53	2256.33
9.	Catalase	Treatments	17	38.67
		Error	36	15.21
		Total	53	53.88
10.	Peroxidase	Treatments	17	202.97
		Error	36	1.90
		Total	53	204.88
11.	DPPH radical scavenging (%)	Treatments	17	3161.21
		Error	36	29.81
		Total	53	3191.02
12.	TSS	Treatments	17	1097.04
		Error	36	10.67
		Total	53	1107.70
13.	Phenylalanine ammonia lyase	Treatments	17	0.01
		Error	36	3.60
		Total	53	0.01
14.	Polyphenol oxidase	Treatments	17	2930.59
		Error	36	36.00
		Total	53	2966.59

*Significance at 5% level of significance

APPENDIX IV

Anova for the quality analysis and sensory analysis of appetizer (experiment 2)

S.No.	Parameters	Source	2017-18	
			d.f.	S.S.
		A	10	14.07
		B	5	45.90
1.	Total sugar	A X B	50	3.43
		Error	132	2.55
		Total	197	65.95
		A	10	6.02
		B	5	31.90
2.	Reducing sugar	A X B	50	8.76
		Error	132	2.32
		Total	197	49.00
		A	10	8.31
		B	5	24.28
3.	Non reducing sugar	A X B	50	3.19
		Error	132	3.98
		Total	197	39.76
		A	10	0.98
		B	5	6.66
4.	Titratable acidity	A X B	50	1.23
		Error	132	1.32
		Total	197	10.19
		A	10	261.44
		B	5	19796.15
5.	Ascorbic acid	A X B	50	43.40
		Error	132	74.25
		Total	197	20175.23
		A	10	8.05
		B	5	0.95
6.	Moisture	A X B	50	5.70
		Error	132	1.86
		Total	197	16.56
		A	10	11.67
		B	5	2.84
7.	Non enzymatic browning	A X B	50	8.30
		Error	132	0.46
		Total	197	23.28
8.		A	10	27.56
		B	5	15.95
	Colour	A X B	50	77.74
		Error	132	74.06
		Total	197	195.30

		A	10	36.03
		B	5	8.33
9.	Flavour	A X B	50	47.09
		Error	132	34.55
		Total	197	126.00
		A	10	62.56
		B	5	3.97
10.	Taste	A X B	50	75.00
		Error	132	55.91
		Total	197	197.43
		A	10	62.78
		B	5	11.61
11.	Texture	A X B	50	41.16
		Error	132	75.05
		Total	197	190.59
		A	10	29.06
		B	5	3.31
12.	Overall acceptability	A X B	50	68.61
		Error	132	48.92
		Total	197	149.90

Significance at 5% level of significance

APPENDIX V

List of chemical preparation, buffer Solutions used for molecular work.

1. DNA extraction buffer (100 ml)

Tris-buffer	:10 ml
0.5M EDTA	:4ml
CTAB 2% (w/v)	:2 g
5M NaCl	:28ml
β-mercapthoethanol	:200µl

Made the volume up to 100ml. autoclaved at 15lb (psi) for 20 min and stored at RT.

2. Isopropanol

It should be store at 4°C in 100 ml dark colored bottle.

3. 70 % ethanol (100 ml)

Absolute Ethyl Alcohol	: 70 ml
Distilled water	: 30 ml

4. TE buffer (pH 8.0, 100ml)

Tris base (1M)	: 1 ml
EDTA (0.5M)	: 200µl

Made up the volume to 100 ml. Autoclaved and stored at room temperature.

5. 5 M Potassium acetate

Potassium acetate	: 49.06gm
Glacial acetic acid	: 11.5ml

Made up the volume to 100 ml. Autoclaved and stored at room temperature.

Solutions for DNA purification

1. Phenol: Chloroform: Isoamyl alcohol (500 ml)

Redistilled phenol	: 250 ml
Chloroform	: 240 ml
Isoamyl alcohol	: 10 ml

Store in the brown bottle or ordinary bottle covered with two folds of silver foil and store at -20°C.

3. 70 % ethanol (100 ml)

Absolute Ethyl Alcohol : 70 ml

Distilled water : 30 ml

4. TE buffer (pH 8.0, 100ml)

Tris base (1M) : 1 ml

EDTA (0.5M) : 200µl

Made up the volume to 100 ml. Autoclaved and stored at room temperature.

5. 5 M Potassium acetate

Potassium acetate : 49.06gm

Glacial acetic acid : 11.5ml

Made up the volume to 100 ml. Autoclaved and stored at room temperature.

Solutions for DNA purification

1. Phenol: Chloroform: Isoamyl alcohol (500 ml)

Redistilled phenol : 250 ml

Chloroform : 240 ml

Isoamyl alcohol : 10 ml

Store in the brown bottle or ordinary bottle covered with two folds of silver foil and store at -20°C.

Stock solutions for gel electrophoresis

DNA loading dye (6X)

For 10 ml

Bromophenol Blue (0.25% w/v) 0.025 g

Glycerol (40%) 4 ml

Dissolved properly and the volume was made up to 10 ml by 1X TAE and stored at 4^0C.

Electrophoresis Buffer (TBE 5X)

For 1000 ml

Tris base 54 g

Boric acid 27.5 g

0.5M EDTA (pH=8) 20 ml

Final pH=8.2 to 8.3

The volume was made up to 1000ml by distilled water and stored at room temperature.

Ethidium bromide (10000X)

Ethidium bromide 10 mg

Distilled water 1 ml

Dissolved properly and stored at 4^0C.

APPENDIX VI

List of Instrument/ Equipments used in the study

Name of instrument	Company/Model
Laminar air flow	Lab Companion,BC-11, Korea
PCR thermocycler	Eppendorf Germany
Refrigerated centrifuge	Sigma (3-18k), Germany
Refrigerated microfuge	Eppendorf, Germany
Water bath	Lab Companion,BW-20G, Korea
Nanodrop	THERMO scientific
Microwave oven	LG,GL-406MQ,India
Magnetic stirrer	BIOSAN,PV-2400, Latavia
Refrigerator	LG, GL-406MQ, India
Deep freezer	Vestfrost,BSF-345, Denmark
Ph meter	EUTECH, Singapore
Digital balance	Precisa, Switzerland
Gel documentation system	Alphamanager, AT 126SL, USA
Agarose gel electrophoresis system	SCIE PLAS, CHU25, UK
Power pack	Consort, EV 215
Digital Spectrophotometer	Biogen scientific BSC-304
HPLC	Agilent 1120 LC, Germany

APPENDIX VII

Performa ForSensory Evaluation of Bael Fruit Appetizer

S.No.	Treatment Combination	Color	Flavor	Taste	Texture	Overall Acceptability

Hedonic scale values

9 Like extremely

8 Like very much

7 Like moderately

6 Like slightly

5 Neither like nor dislike

4 Dislike slightly

3 Dislike moderately

2 Dislike very much

1 Dislike exteraemely

APPENDIX VIII

Economics of various treatments combinations of Bael genotype and ingredients for 1kg appetizer preparation (5 packets of 200gm)

Characteristic	Cost of Bael fruit	Total cost of ingredients	Processing cost	Total cost	Assumed product value (1 packets of 200gm)	Assumed product value (5 packets of 200gm)	Gross return	Net return	Benefit: cost ratio
AB1(100% Bael powder)	160	0	20	180	100	500	500	320.00	1.78:1
AB2 (96% Bael powder + 3% Black salt + 1% Black pepper)	160	10.15	20	190.15	105	525	525	334.85	1.76:1
AB3 (48% Bael powder + 48% Fenugreek seed powder+ 3% Black salt + 1% Black pepper)	80	71.50	20	171.5	130	650	650	478.50	2.79:1
AB4 (48% Bael powder + 48% Aonla+ 3% Black salt + 1% Black pepper	80	71.50	20	171.5	130	650	650	478.50	2.79:1
AB(48% Bael powder + 48% Jamun powder+ 3% Black salt + 1% Black pepper	80	111.5	20	211.5	180	900	900	688.50	3.26:1
AB6 (24% Bael powder + 24% Fenugreek powder + 24% Aonla fruit powder + 24% Jamun seed powder + 3% Black salt + 1.5% Black pepper)	32	121.5	20	171.5	200	1000	1000	828.50	4.83:1

*A= Any genotype of Bael, *B= Different ingredients

VITAE

Ms. Anjana Kholia, the authoress of this manuscript was born on June 14, 1991, at Pithoragarh (Uttarakhand). She passed her High School and Intermediate examination from Uttrakhand Board with first division in 2007 and 2009, respectively. After completing graduation in first division from G.B. Pant University of Agriculture and Technology in 2013, Pantnagar, Distt. U.S. Nagar, she joined Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra for M.Sc. Horticulture (Fruit Science) Degree programme and completed her degree in first division with distinction in 2015. Further she joined Ph. D. with major in Horticulture and minor in Molecular Biology and Biotechnology in July, 2015 in G.B. Pant University of Agriculture and Technology, Pantnagar Distt. U.S. Nagar and completed her degree requirement in July, 2018. She has published 5 research papers and received one best oral presentation award in national conference. She had been a recipient of University fellowship during her Ph. D. programme.

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Minor	: Molecular Biology and Biotechnology		
Thesis Title	: "Biochemical and molecular characterization of diverse genotypes of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties"		
Advisor	: Dr. K.K. Misra		

ABSTRACT

The investigation entitled "Biochemical and molecular characterization of diverse genotypes of Bael (*Aegle marmelos* Correa.) for their nutraceutical properties" was carried out on 24 diverse genotypes of Bael at G. B. Pant University of Agriculture & Technology, Pantnagar, U. S. Nagar, Uttarakhand, during the year 2016-18.

Out of the 24 genotype , the 18 genotypes of Bael were characterized on the basis of biochemical parameters *i.e.,* ascorbic acid, marmelosin, total phenol content, pectin content, riboflavin, total carotenoids, total falvonoids, enzymatic antioxidants, DPPH radical scavenging activity, total soluble sugar, phenylalanine ammonia lyase and polyphenol oxidase enzyme activity. The biochemical analysis revealed that Pant Bael-1, Pant Sujata, Pant Bael-3, Pant Shivani and Pant Aparna were found suitable for future breeding programme as they had more than one desirable biochemical character. The 24 genotypes of Bael were characterized on the basis of SSR marker based polymorphism. The UPGMA dendrogram based on genetic distance, segregated the 24 Bael genotypes into two major clusters. The major cluster A consist of 19 genotypes, which was further divided into two sub clusters IA and IIA at 66 per cent similarity. The major cluster B consist of 5 genotypes, which was further divided into two sub cluster IB and IIB at 78 per cent similarity. The polymorphic Information Content (PIC) values ranged from 0.234-0.998. Jaccard's similarity coefficient values ranged from0.271 to 0.951with polymorphism of 100 per cent. A total of 59 loci were detected with amplified size range of 100 to 800 bp. The maximum numbers of loci (5) were detected by the primer CAC33. The 11 genotypes of Bael with 66 treatment combination were used to develop the nutraceuticaly rich appetizer. The quality analysis was done on the basis of total sugar, reducing sugar, non reducing sugar, titratable acidity, ascorbic acid, moisture and non enzymatic browning of appetizer. The sensory evaluation of appetizer was done based on different sensory parameters *i.e.,* colour, flavour, taste, texture and over all acceptability. The highest score obtained for different sensory parameters was 9 (Like extremely) and the lowest score was 5 (Neither like or nor dislike).

The significant variation exist among the genotypes based on biochemical characters but with the use of SSR markers, assessment of the genetic diversity can also help us to plan a future breeding programme using the diverse parent. The nutraceutically rich appetizers were developed involving different Bael genotypes and ingredients.

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(K.K. Misra)
Advisor

Anjana
(Anjana Kholia)
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नाम	: अंजना खोलिया	**परिचयांक**	: 38105
सत्र एवं प्रवेश का वर्ष	: प्रथम, 2015–16	**उपाधि**	: पी.एचडी.
मुख्य विषय	: उद्यान विज्ञान	**विभाग**	: उद्यान विज्ञान
गौण विषय	: आणविक जीव विज्ञान और जैव प्रौद्योगिकी		
शोध शीर्षक:	''बेल (*एग्ल मार्मलास* कोरिया) के विभिन्न जीनोटाइप्स का नूट्रास्यूटिकल गुणों के लिए जैव–रासायनिक एवं आणविक चरित्रीकरण''		
सलाहकार	: डॉ0 के0के0 मिश्र		

सारांश

प्रस्तुत अध्ययन ''बेल के विभिन्न जीनोटाइप्स का नूट्रास्यूटिकल गुणों के लिए जैव–रासायनिक एवं आणविक चरित्रीकरण'' गो0ब0 पंत कृषि एवं प्रौद्योगिकी विश्वविद्यालय, पंतनगर, उत्तराखण्ड में 2016–18 में बेल के चौबिस (24) जीनोटाइप्स में किया गया। कुल चौबिस में से अट्ठारह (18) जीनोटाइप्स का चरित्रीकरण जैव–रासायनिक मानकों जैसे कि एस्कॉर्बिक ऐसिड, मारमेलोसिन, कुल फिनॉल, पेक्टिन, राइबोफ्लेविन, कुल केरोटेनोइड्स, कुल फ्लेवनॉइड्स, ऐंजाइमैटिक एंटीऑक्सिडेंट्स, डीपिपिअच, रेडिकल स्केवेंजिंग एक्टिविटी, टी.एस.एस., फिनाइल अलनीन अमोनिआलेज एवं पॉलीफेनॉल ऑक्सिडेस एंजाइम एक्टिविटी के आधार पर किया गया। अट्ठारह (18) जीनोटाइप्स के जैव–रासायनिक गुणों के अध्ययन से ये ज्ञात हुआ कि पंत बेल–1, पंत सुजाता, पंत बेल–3, पंत शिवानी तथा पंत अपर्णा में एक से अधिक वांछित गुण पाये गए। अतः भविष्य में फसल प्रजनन कार्य की योजना के लिए इनका उपयोग किया जा सकता है। कुल चौबिस जीनोटाइप्स का एस.एस.आर. मार्कर्स आधारित बहुरूपता के आधार पर चरित्रीकरण किया गया। यूपीजीएमए समूह विश्लेषण के आधार पर चौबिस जीनोटाइप्स को दो प्रमुख समूहों – समूह–ए और समूह–बी में बाँटा गया। प्रमुख समूह–ए जिसमें उन्नीस जीनोटाइप्स थे, को छियासठ प्रतिशत (66%) समानता के आधार पर पुनः दो उप–समूहों ए$_1$ एवं ए$_2$ में बाँटा गया। प्रमुख समूह–बी जिसमें पांच (5) जीनोटाइप्स थे, को अठहत्तर प्रतिशत (78%) समानता के आधार पर पुनः दो उप–समूहों बी$_1$ एवं बी$_2$ में बाँटा गया। बहुरूपता संकेतन मात्रा (पी.आई.सी.) का मान 0.234–0.998 पाया गया। जकार्ड एकरूपता गुणांक का मान सौ प्रतिशत बहुरूपता के साथ 0.271–0.951 पाया गया। कुल उनसठ बिन्दुपथ प्राप्त हुए जिनके चिन्हित विकल्प का मान 100–800 बीपी की सीमा के मध्य था। प्राइमर CAC33 के लिए अधिकतम पांच (5) बिन्दुपथ प्राप्त हुए।

जैव–रासायनिक एवं आणविक चरित्रीकरण के अलावा, बेल के ग्यारह (11) जीनोटाइप्स और छियासठ (66) उपचार संयोजन का प्रयोग नूट्रास्यूटिकल–सम्पन्न क्षुधावर्धक बनाने में किया गया। क्षुधावर्धक की गुणवत्ता का अध्ययन टोटल शुगर, रीड्रसिंग शुगर, एसिडिटी, एस्कॉर्बिक एसिड, नमी एवं नॉन एंजाइमेटिक ब्राउनिंग के आधार पर किया गया। क्षुधावर्धक की ग्रहणशीलता का अध्ययन विभिन्न ग्रहणशील मानकोंः रंग, महक, स्वाद, संरचना तथा समग्र स्वीकार्यता के आधार पर किया गया। विभिन्न ग्रहणशील मानकों के लिए सर्वाधिक अंक नौ (9) और निम्नतम् अंक पाँच (5) पाया गया। बेल के विभिन्न जीनोटाइप्स में जैव–रासायनिक अध्ययन के आधार पर स्पष्ट विभिन्नता उपस्थित पाए गए, लेकिन अनुवांशिक विविधता एस.एस.आर. मार्कर्स के माध्यम से विविध पैतृक जीनोटाइप्स का उपयोग कर भविष्य में फसल प्रजनन कार्य की योजना बनाये जा सकती हे, साथ ही बेल के विभिन्न जीनोटाइप्स एवं सामग्री को मिलाकर नूट्रास्यूटिकल–सम्पन्न क्षुणावर्धक बनाने में किया जा सकता है।

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