A STUDY ON ANTHOCYANINS AND OTHER BIOCHEMICAL CONSTITUENTS IN DIFFERENT PLANT PARTS OF BRINJAL (*Solanum melongena* L.)

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
BIOCHEMISTRY
(Minor Subject: Biotechnology)

By

Ankita Kumari
(L-2012-BS-208-M)

Department of Biochemistry
College of Basic Sciences and Humanities

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CERTIFICATE – I

This is to certify that the thesis entitled, **"A study on anthocyanins and other biochemical constituents in different plant parts of brinjal"**, submitted for the degree of **M.Sc** in the subject of **Biochemistry** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana is a bonafide research work carried out by **Ankita Kumari (L-2012-BS-208-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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Major Advisor
(Dr. (Mrs.) Neena Chawla)
Senior Biochemist
Department of Vegetable Science,
Punjab Agricultural University,
Ludhiana – 141004 (India)

CERTIFICATE - II

This is to certify that the thesis entitled **"A study on anthocyanins and other biochemical constituents in different plant parts of brinjal"** submitted by **Ankita Kumari (L-2012-BS-208-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of M.Sc., in the subject of **Biochemistry** (Minor subject: **Biotechnology**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

_____

(Dr. (Mrs.) Neena Chawla)
Major Advisor

_____

(Dr. S.K. Munshi)
External Examiner
Professor
Deptt. of Biotechnology
PCTE, Jhande
Ludhiana

_____

(Dr. Bavita Asthir)
Head of the Department

_____

(Dr. Gursharan Singh)
Dean, Post-Graduate Studies

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ABSTRACT

Fifty genotypes of brinjal bearing fruits of different colours (purple, pink, green and white) were evaluated for anthocyanin content in peel, flesh part and whole fruit in fresh tissue, whole fruits were analyzed for dry matter, total soluble sugars, total phenols, ortho-dihydroxy phenols and flavonols in 2012 and 2013. In both years, the peel had high anthocyanin content followed by whole fruit and flesh part. The highest anthocyanin content in peel, flesh and whole fruit was found in SR-312 (purple), SR-308 (green) and SR-303 (purple) respectively. Total phenols content varied in the range of 70.21 to 296.37 mg/100g DW with average value of 125.58 mg/100g in 2012 and in the range of 75.16 to 298.63 mg/100g DW with average value of 120.78 mg/100g in 2013. Highest total phenol content was found in G-415 (green) in 2012 and 2013. G-418 (green) and P-71 (purple) showed ortho-dihydroxy phenols content in the maximum range (80-100 mg/100g). The maximum flavonol content was found in BLEND-11-WR-2 (white). The activity of three enzymes ANS, PAL and TAL were studied in fruit and leaves of fourteen selected genotypes. The activity of ANS in leaves and fruits of green and white colour progressively decreased from 7 days to 21 days while a progressive increase was observed in purple fruits during the same period. The activity of PAL and TAL in both fruit and leaves increased till 14 days and then there was decrease in activity upto 21 days.

Keywords: anthocyanins, brinjal, total phenols, ANS, PAL, TAL

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ਸਾਰੰਸ਼

ਸਾਲ 2012 ਅਤੇ 2013 ਵਿੱਚ ਵੱਖੋ-ਵੱਖ ਰੰਗਾਂ (ਜਾਮਨੀ, ਗੁਲਾਬੀ, ਹਰੇ ਅਤੇ ਚਿੱਟੇ) ਦੇ ਬੈਂਗਣਾਂ ਦੀਆ 50 ਕਿਸਮਾਂ ਦੇ ਛਿਲਕੇ, ਛਿਲਕਾ-ਰਹਿਤ ਭਾਗ ਅਤੇ ਪੂਰੇ ਫ਼ਲ ਦਾ ਐਂਥੋਸਾਇਆਨੀਨ ਲਈ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ । ਪੂਰੇ ਫ਼ਲ ਦਾ ਮੁਲਾਂਕਣ ਸੁੱਕੇ ਮਾਦੇ, ਕੁੱਲ ਸ਼ੁਗਰ, ਕੁੱਲ ਫ਼ੀਨੋਲ ਅਤੇ ਫ਼ਲੇਵਾਨੋਲ ਨਾਮਕ ਤੱਤਾਂ ਲਈ ਦੋਹਾਂ ਸਾਲਾਂ ਵਿੱਚ ਕੀਤਾ ਗਿਆ । ਸਾਲ 2012 ਅਤੇ 2013 ਵਿੱਚ ਛਿਲਕੇ ਵਿੱਚ ਐਂਥੋਸਾਇਆਨੀਨ ਦੀ ਮਾਤਰਾ ਸਭ ਤੋਂ ਵੱਧ ਪਾਈ ਗਈ, ਪੂਰੇ ਫ਼ਲ ਵਿੱਚ ਉਸ ਤੋਂ ਘੱਟ ਅਤੇ ਛਲਿਕਾ-ਰਹਿਤ ਭਾਗ ਵਿੱਚ ਐਂਥੋਸਾਇਆਨੀਨ ਦੀ ਸਭ ਤੋਂ ਘੱਟ ਮਾਤਰਾ ਵੇਖੀ ਗਈ । ਛਿਲਕੇ, ਛਿਲਕਾ-ਰਹਿਤ ਭਾਗ ਅਤੇ ਪੂਰੇ ਫ਼ਲ ਵਿੱਚ ਐਂਥੋਸਾਇਆਨੀਨ ਦੀ ਵਧੇਰੇ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ ਐਸ.ਆਰ.-312 (ਜਾਮਨੀ), ਐਸ.ਆਰ.-308 (ਹਰਾ) ਅਤੇ ਐਸ.ਆਰ.-303 (ਜਾਮਨੀ) ਵਿੱਚ ਪਾਈ ਗਈ। ਕੁੱਲ ਫ਼ੀਨੋਲ ਦੀ ਮਾਤਰਾ 2012 ਵਿੱਚ 70.02 ਤੋਂ 296.37 ਮਿਲੀਗ੍ਰਾਮ ਪ੍ਰਤੀ 100 ਗ੍ਰਾਮ ਅਤੇ 2013 ਵਿੱਚ 75.16 ਤੋਂ 298.63 ਮਿਲੀਗ੍ਰਾਮ ਪ੍ਰਤੀ 100 ਗ੍ਰਾਮ ਸੀ । ਜਦੋਂ ਕਿ ਦੋਹਾਂ ਸਾਲਾਂ ਵਿੱਚ ਇਸ ਤੱਤ ਦੀ ਔਸਤ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ 125.58 ਅਤੇ 120.78 ਮਿਲੀਗ੍ਰਾਮ ਪ੍ਰਤੀ 100 ਗ੍ਰਾਮ ਸੀ । ਵਰਣਨਯੋਗ ਹੈ ਕਿ ਦੋਹਾਂ ਸਾਲਾਂ ਵਿੱਚ ਕੁੱਲ ਫ਼ੀਨੋਲ ਜੀ-415 (ਹਰੇ) ਵਿੱਚ ਸਭ ਤੋਂ ਉਚੇਰੀ ਮਾਤਰਾ ਵਿੱਚ ਸਨ । ਜਿਨਸਾਂ ਜੀ-418 (ਹਰੇ) ਅਤੇ ਪੀ-71 (ਜਾਮਨੀ) ਵਿੱਚ ਆਰਥੋਡਾਈਹਾਈਡਰੌਕਸੀ-ਫ਼ੀਨੋਲ (80 ਤੋਂ 100 ਮਿਲੀਗ੍ਰਾਮ ਪ੍ਰਤੀ 100 ਗ੍ਰਾਮ) ਅਤੇ ਬਲੈਂਡ 11-ਡਬਲਿਊ-ਆਰ-2 (ਚਿੱਟੇ) ਵਿੱਚ ਫ਼ਲੇਵਾਨੋਲ ਨਾਮਕ ਤੱਤ ਸਭ ਤੋਂ ਵੱਧ ਸਨ । 14 ਚੌਣਵੀਆਂ ਜਿਨਸਾਂ ਦੇ ਫ਼ਲਾਂ ਅਤੇ ਪੱਤਿਆਂ ਵਿੱਚ ਤਿੰਨ ਐਂਜ਼ਾਈਮਾਂ (ਐਂਥੋਸਾਇਆਨੀਡੀਨ ਸੀਂਥੇਜ਼, ਫ਼ੀਨਾਈਲਐਲਾਨੀਨ ਅਮੋਨੀਆਂ ਲਾਈਏਜ਼ ਅਤੇ ਟਾਇਰੋਸੀਨ ਅਮੋਨੀਆਂ ਲਾਈਏਜ਼) ਦੀ ਗਤੀਵਿਧੀ ਦੀ ਘੋਖ ਕੀਤੀ ਗਈ । ਚਿੱਟੀਆਂ ਅਤੇ ਹਰੀਆਂ ਕਿਸਮਾਂ ਦੇ ਫ਼ਲ ਅਤੇ ਪੱਤਿਆਂ ਵਿੱਚ ਐਂਥੋਸਾਇਆਨੀਡੀਨ ਸੀਂਥੇਜ਼ ਦੀ ਗਤੀਵਿਧੀ 7 ਤੋਂ 21 ਦਿਨ ਤੱਕ ਲਗਾਤਾਰ ਘੱਟਦੀ ਰਹੀ, ਜਦਕਿ ਜਾਮਨੀ ਕਿਸਮ ਦੇ ਫ਼ਲਾਂ ਵਿੱਚ ਇਹ ਲਗਾਤਾਰ ਵੱਧਦੀ ਪਾਈ ਗਈ। ਜਾਮਨੀ ਕਿਸਮ ਦੇ ਪੱਤਿਆਂ ਵਿੱਚ ਐਂਥੋਸਾਇਆਨੀਡੀਨ ਸੀਂਥੇਜ਼ ਦੀ ਗਤੀਵਿਧੀ 7 ਤੋਂ 21 ਦਿਨਾਂ ਤੱਕ ਘੱਟਦੀ ਵੇਖੀ ਗਈ। ਦੂਜੇ ਪਾਸੇ, ਫ਼ੀਨਾਈਲਐਲਾਨੀਨ ਅਮੋਨੀਆ ਲਾਈਏਜ਼ ਅਤੇ ਟਾਇਰੋਸੀਨ ਅਮੋਨੀਆਂ ਲਾਈਨੇਜ਼ ਦੀ ਗਤੀਵਿਧੀ ਪੱਤਿਆਂ ਅਤੇ ਫ਼ਲਾਂ ਵਿੱਚ 14 ਦਿਨ ਤੱਕ ਚੜ੍ਹਤ ਤੇ ਰਹੀ, ਪਰ ਉਸ ਤੋਂ ਬਾਦ 21 ਦਿਨ ਤਕ ਲਗਾਤਾਰ ਘੱਟਦੀ ਪਾਣੀ ਗਈ ।

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ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

LIST OF ABBREVIATIONS

%	=	Percentage
δ	=	Delta
µmoles	=	micromoles
µg	=	microgram
µM	=	micromolar
β	=	Beta
˚C	=	Centigrade
A	=	Absorbance
AOAC	=	Association of Official Analytical Chemists
ANS	=	Anthocyanidin synthase
BC	=	Before Christ
BSA	=	Bovine serum albumin
Chl	=	Chlorophyll
conc.	=	concentrated
D	=	Day
DAA	=	Days after anthesis
DHQ	=	dihydroquercetin
Fig	=	Figure
FW	=	Fresh weight
g	=	gram
h	=	hour
ha	=	hectare
HCl	=	Hydrochloric Acid
kcal	=	kilocalorie
M	=	Molar
mg	=	milligram
min	=	minute
ml	=	millilitre
mM	=	millimolar
MT	=	Metric tonne
N	=	Normal
nm	=	nanometer
o	=	ortho
ODH	=	Ortho-dihydroxy phenol
p	=	para
PAL	=	Phenylalanine ammonia lyase
PVP	=	Polyvinyl pyrrolidine
rpm	=	Revolution per minute
S D	=	Standrad Deviation
S E	=	Standard Error
TAL	=	Tyrosine ammonia lyase
TCA	=	Trichloroacetic acid
USDA	=	United States Department of Agriculture
UV	=	Ultra-Violet
v	=	Volume
VIS	=	Visible
w	=	weight

CHAPTER I

INTRODUCTION

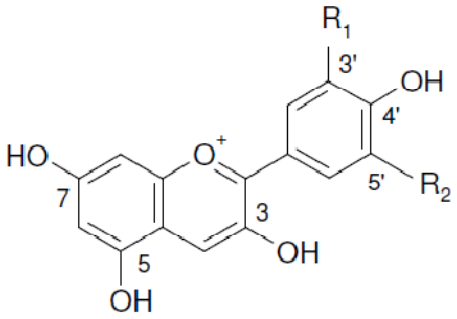
Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of sub-tropics and tropics. The name brinjal is popular in Indian subcontinents and is derived from Arabic and Sanskrit whereas the name eggplant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken eggs. It is also called aubergine (French word) in Europe.

The brinjal is of much importance in the warm areas of Far East, grown extensively in India, Bangladesh, Pakistan, China and the Philippines. It is also popular in Egypt, France, Italy and the United States. In India, it is one of the most common and popular vegetable crop grown throughout the country except at higher altitudes. Eggplant exhibits wide diversity in growth habit, vegetative characters and floral morphology and produces fruit with many different shapes, sizes and colours, depending on the cultivar. It is adapted to different agro-climatic regions and can be grown throughout the year (Cork *et al* 2005). A temperature of 13˚ to 21˚C is most favourable for optimum growth and yield (Kalloo *et al* 1990, Kumar *et al* 2000, Mohanty and Prusty 2000, Thapa 2002). India is the second largest producer of brinjal after China. The crop covers an area of 7.2 million ha with an annual production of 134.4 million MT with an average yield of 18.6 MT/ha in India. Punjab produces 81.27 thousand tonnes of brinjal in the area of 3.8 thousand ha with an average yield of 21.3 MT/ha (National Horticulture Board 2013).

Eggplant or brinjal has a very low calorie value and a healthy nutrient profile. According to USDA Nutrient Data Base, 100 g of eggplant contains energy (24 kcal), carbohydrates (5.7 g), fat (0.19 g), protein (1.01 g), thiamine (0.039 mg), riboflavin (0.037 mg), niacin (0.649 mg), pantothenic acid (0.281 mg), vitamin B6 (0.084 mg), folate (0.22 mg), vitamin C (2.2 mg), calcium (9 mg), iron (0.24 mg), magnesium (14 mg), manganese (0.25 mg), phosphorus (25 mg), potassium (230 mg) and zinc (0.16 mg). The unripe fruit of brinjal is primarily used as a cooking vegetable for the various dishes in different regions of the world. It has much potential as raw material in pickle making and dehydration industries. Brinjal is known to have ayurvedic medicinal properties. It is said to be good for diabetic patients (Choudhury 1976). It has been recommended as an excellent remedy for liver problems (Shukla and Naik 1993).

Phenolic compounds form one of the main classes of secondary metabolites. Among these compounds, flavonoids are important group of plant phenolics. So far, over 8000 varieties of flavonoids have been identified. These perform various roles in plants such as UV protection, defence against pathogens and pests, pollen fertility, signalling with

microorganisms, regulation of auxin transport and pigmentation (Winkel-Shirley 2001). Anthocyanins are the most important class of flavonoids. Anthocyanins are water-soluble natural pigments which give pink, red, magenta, purple and dark blue colours to flowers and fruits depending on pH. These pigments are synthesized in the cytosol and are accumulated in the vacuoles of plant cells. These are mainly present in epidermal cells and also in flesh. The accumulation of anthocyanins varies with plant species, cultivar, tissue structure, geographical location and cultivation conditions. They are produced by secondary metabolism of plants (pentosephosphate, shikimate and flavonoid pathways). They are odourless and nearly flavourless, contributing to taste as moderately astringent sensation. Anthocyanins occur in all tissues of higher plants including leaves, stems, roots, flowers and fruits. *Vaccinium* species such as blackberry, blueberry, eggplant peel, black rice are rich in anthocyanins. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. The difference between individual anthocyanins relates to the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of their attachment and the nature and number of aliphatic or aromatic acids attached to sugars in the molecule.



R_1 = H, R_2 = H, pelargonidin
R_1 = OH, R_2 = H, cyanidin
R_1 = OH, R_2 = OH, delphinidin
R_1 = OCH_3, R_2 = H, peonidin
R_1 = OCH_3, R_2 = OH, petunidin
R_1 = OCH_3, R_2 = OCH_3, malvidin

Fig 1: Basic structure of anthocyanidin.

Various factors such as pH, temperature, UV radiations, ascorbic acid and chelating metal ions etc govern the stability of anthocyanins. The change in these factors results in colour change and anthocyanin degradation (Rivas-Gonzalo 2003). The most significant function of anthocyanins is their ability to impart colour to the plants or plant products in which they occur.

Condensed tannis (CTs) also known as proanthocyanidins (Pas) are the polymers of flavonoids molecules. Tannins are present in plant seeds and flesh and becomes brown from colourless on oxidation. Tannins impart astringent quality to the developing fruit which deter animals from feeding (Feeny 1970, Forkner *et al* 2004). Lignins which are polymers of 4-hydroxyphenylpropanoids are also derived from the phenylpropanoid pathway (Boerjan *et al* 2003). Lignins have role in plant development and resistance to biotic and abiotic stresses because the polymers confer strength and rigidity to the cell wall. Flavonols are required for

functional pollen in maize and petunia (Deboo *et al* 1995) and also play an important role in UV photoprotection (Lois and Buchanan 1994, Ryan *et al* 2002). Flavonols also influence anthocyanin colour via a co-pigmentation in the vacuole (Lancaster 1992, Fossen *et al* 2000). Flavonoids and phenolic acids are very effective free radical scavengers (Nakatani *et al* 2000). They protect the plants from damage caused by UV radiations, attract pollinators and other beneficial organisms or mediate plant-microbe interactions (Buer *et al* 2010). Along with other flavonoids, anthocyanins are involved in the resistance of plants to insect attack (Harborne 1988).

Plant phenolic phytochemicals have several potential health promoting effects (Kinsella *et al* 1993). These have an important role in the prevention of lipid peroxidation of cell membranes which is induced by active oxygen radicals in living systems. Consumption of polyphenols reduces radical-mediated pathogeneses such as carcinogenesis and atherosclerosis (Ames *et al* 1993, Sawa *et al* 1999). Anthocyanins are known to enhance the anti-ulcer activity (Cristoni and Magistretti 1987), sight acuteness, the maintenance of normal vascular permeability (Lamer-Zarawska and Oszmianski 1991,1994) *in vitro* inhibition of platelet aggregation (Tamas *et al* 2000), protection against liver damage (Wang *et al* 2000), antimutagenic activity (Hope Smith *et al* 2004), stimulation of insulin in pancreatic cells (Jayaprakasam *et al* 2005), anti-tumor activity (Liu *et al* 2005) and may prevent hyperlipidemia (Xia *et al* 2007) and cardiovascular diseases (Gracia-Alonso *et al* 2004). Due to these medicinal properties, anthocyanin extracts can be used as components of pharmaceutical preparations and functional foods. Due to a variety of pharmacological activities in mammalian body, anthocyanins are referred to as "Nutraceuticals".

Colour is one of the most important properties of food and beverages. Characteristic colour is the basic property for identification and acceptance of many foods and beverages. Colour also represents an important attribute for marketing purposes (Clifford 2000, Hardy 2000). Synthetic colouring agents have commonly been used in the food industry. However, the safety of synthetic dyes is questioned which has led to a reduced use of these in many edible items. This has increased the interest in natural colorants (Bakowska-Barczak 2005). Anthocyanins have shown a high potential to be used as natural colorants due to their attractive orange, red and purple colours and water solubility (Brouillard 1982, Mazza and Miniati 1993, Shahidi and Naczk 1995). Acetylated anthocyanins are suitable to be applied for foods with a low pH level such as soft drinks, confectionary, table jellies, sauces and water ice-cream.

Eggplant or brinjal has high oxygen radical absorbance capacity (ORAC) (Cao *et al* 1996). It is a rich source of antioxidants such as flavonoids and phenolic acids (Mennella *et al* 2010, Stommell and Whitaker 2003). These compounds are present in both the fruit's flesh and skin (Huang *et al* 2004) and their content is regulated during fruit ripening (Mennella *et*

al 2012). Nasunin is a major anthocyanin found in peel of purple brinjal but not in white and green and has been reported to contain high antioxidant activity (Igarashi *et al* 1993, Noda *et al* 2000).

Brinjal has high anthocyanin content which provides a promising alternative to synthetic colorants in food systems and also has high phenolic content which can contribute to various therapeutic potential health effects. These pharmaceutical uses of brinjal have not been exploited much in India. Present study was planned with the following objectives:

Objectives:

- To screen brinjal genotypes for anthocyanins, phenolics and other components.
- To study the enzymes for anthocyanin synthesis in selected genotypes.

CHAPTER II

REVIEW OF LITERATURE

Eggplant is an Old World crop of solanaceous family. Sanskrit documents have revealed that the domestication of eggplant was achieved as early as 100-300 BC. Archaeological records, based on the analysis of microfossils suggested that eggplant was present in the diet of inhabitants of the Indus valley during Harappan civilisation, thus Rajasthan might be the area of domestication (Kashyap 2010). The use of eggplant as a vegetable crop was described in Chinese literature dating to 59 BC (Wang *et al* 2008). The crop spread westwards to Persia by the ancient Greeks and Romans and was then introduced to the Mediterranean Basin by Muslim invaders in the 7th to 8th century (Daunay 2008).

The scientific name *Solanum melongena* for brinjal is derived from a 16th century Arabic term. The name brinjal developed in the United States, Australia and Canada. Some 18th century European cultivars were yellow or white and resembled goose or hen's egg hence brinjal was also named as "Eggplant". The name Aubergine in English is based on the French word aubergine. In Indian and South African English the fruit is known as "Brinjal". The fruit is called Vatiganah in Sanskrit and Baingan in Hindi and Urdu. At one time brinjal was believed to be poisonous because of its relationship with the Solanaceae (nightshade) family. While it is generally eaten by most people without any ill effects, it can cause or worsen arthritis. Bajaj *et al* (1979) reported that on an average, the oblong-fruited eggplant cultivars are rich in total soluble sugars, whereas the long-fruited cultivars contain a higher content of free reducing sugars, anthocyanins, phenols, glycoalkaloids (such as solasodine), dry matter, and amide proteins.

The present studies were undertaken to investigate the presence of anthocyanins and other biochemical components in different cultivars of brinjal and their accumulation in different plant parts at different time intervals during plant development. Pertinent literature is reviewed under the following head:

2.1 Anthocyanins

2.1.1 Anthocyanins as colouring agent

2.1.2 Anthocyanins of brinjal

2.2 Enzymes

2.2.1 Anthocyanidin synthase (ANS)

2.2.2 Phenylalanine ammonia lyase (PAL)

2.2.3 Tyrosine ammonia lyase (TAL)

2.3 Other phenolic compounds

2.1 Anthocyanins

It is estimated that more than 635 anthocyanins have been found in nature which are

constituted by anthocyanidins which are glycosylated and acylated differentially (He and Guisti 2010). Hedin *et al* (1983) showed that cyanidin-3-glucoside protects cotton leaves against the tobacco budworm. In addition to their functions in plants, anthocyanins have many other uses. Anthocyanins also possess known pharmacological properties and are used for therapeutic purposes. Wagner (1985) found that pigment extracts were more effective than O-(β-hydroxyethyl) rutin in decreasing capillary permeability and fragility and in their anti-flammatory and anti-edema activities. Cristoni and Magistretti (1987) reported the anti-ulcer activity of anthocyanins. Hertog *et al* (1993 a, b) evaluated that the fruit part contains higher content of anthocyanins as compared to other flavonoids. The anthocyanin content of fruits such as blackberry, blueberry, cranberry, raspberry, strawberry and boysenberry may range from 200 to 4950 mg per kg of fresh fruit. It is 10 to 200 times more than the quercetin content in blue berries. Tsuda *et al* (1996) studied the antioxidative, radical scavenging and inhibitory effects on lipid peroxidation of UV light irradiation of three anthocyanin pigments: pelarogonodin 3-O-beta-D-glucoside (P3G), cyanidin 3-O-beta-D-glucoside (C3G), and delphinidin 3-O-beta-D-glucoside (D3G) isolated from the *Phaseolus vulgaris* L. seed coat. They found that all the pigments had strong antioxidative activity in a liposomal system and reduced the formation of malondialdehyde from UV B (320-290 nm) irradiation. While, Koide *et al* (1996) found that hydrolyzed anthocyanidins contained in grape rinds and red rice gave an elevation of S-phase, which suggested a block in the step from S-phase to G2-phase in HCT-15 cells. Rice-Evans *et al* (1996) and Kim and Lee (2004) showed that the anthocyanins scavenging activity of free radicals has been related to the catechol function of the B-ring. Koide *et al* (1997) also reported the anti-tumor effects *in vitro* and *in vivo* of extracts from red soyabeans, which were composed of mostly cyanin conjugated with glucose and rhamnose. Wang *et al* (1997) and Stintzing *et al* (2002a) suggested that free radical scavenging may also depend on type and extent of glycosylation and acylation respectively. Ghiselli *et al* (1998) reported that the anthocyanin fraction from red wine was the most effective both in scavenging reactive oxygen species and in inhibiting lipoprotein oxidation and platelet aggregation. They found that the inhibition of oxidation increased with the concentration of the antioxidants. Kamei *et al* (1998) showed that anthocyanin fraction from red wine suppressed the growth of HCT-15 cells, derived from human colon cancer or AGS cells derived from human gastric cancer. The flavonoids synthesis pathway is one of the most investigated pathways (Fig. 2). Obi *et al* (1998) examined the ability of anthocyanins obtained from the petals of *H. rosainensis* to prevent carbon tetrachloride-induced acute liver damage in rats. Joseph *et al* (1999) reported that anthocyanins from fruit extracts were effective in reversing age-related deficits. Sarma and Sharma (1999) studied the inter-reaction of anthocyanins and DNA and suggested that the cyanidin-DNA co-pigmentation may be a possible defence mechanism against the oxidative damage to DNA and may have *in vivo*

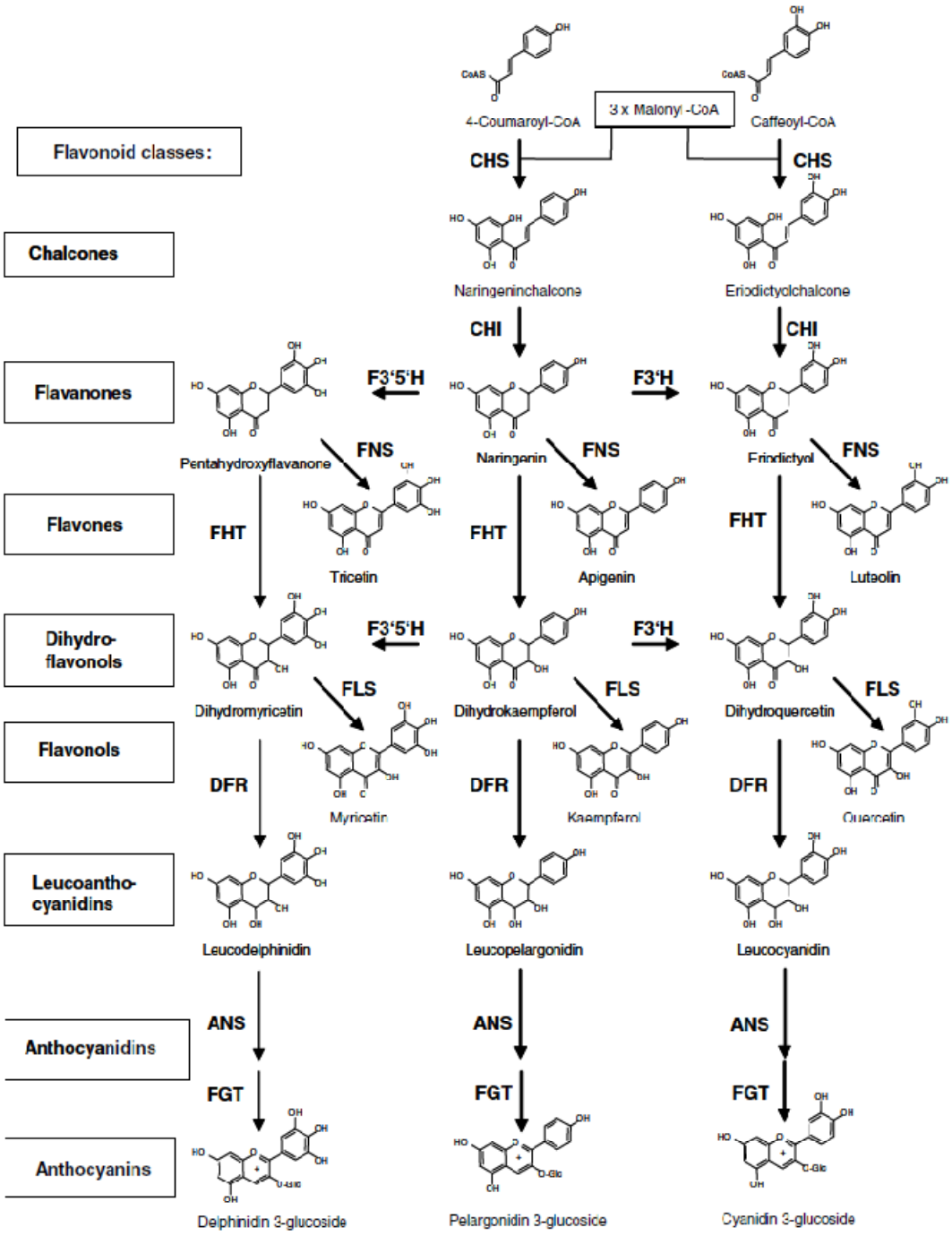


Fig 2: Biosynthesis of important anthocyanins and co-pigments. Abbreviations for the enzymes are as follows: CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase; FHT, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavanoid 3',5'-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; FGT, flavonoid 3-O-glucosyltransferase.

physiological functions attributable to the antioxidant ability of anthocyanins. Yoshimoto *et al* (1999) studied the antimutagenicity of water extracts from the four varieties of sweet potatoes with different flesh colours using Salmonella typhimurium TA 98 and found that anthocyanin pigments from purple coloured sweet potato inhibited the reverse mutation induced by heterocyclic amines-mutagen, Trp-P-1, Trp-P-2 and IQ in the presence of rat liver microsomal activation systems. Deguchi *et al* (2000 a, b) reported radical scavenging activity of hordeumin which is a type of anthocyanin-tanin pigment produced from barley bran-fermented broth. The hordeumin scavenged superoxide radicals in a concentration-dependent manner also decreased reverse mutation of dimethyl sulfoxide extracts from grilled beef. Anthocyanins extracted from black chokeberry, black-thorn and strawberry showed an antiradical capacity (Espin *et al* 2000). Jankowski *et al* (2000 a, b) showed that a simultaneous daily administration of anthocyanins obtained from cabernet red wine and streptozocin substantially decreased sugar concentration in the urine and blood serum. Mas *et al* (2000) suggested that anthocyanins have the ability to stabilize DNA triple-helical complexes. Purple corn colour (PCC) which is a natural anthocyanin was used to modify colorectal carcinogenesis in male F344/duCrj rats that were initially treated with 1, 2-dimethylhydrazine (DMH) by Hagiwara *et al* (2001). Li *et al* (2001) studied the regulation of anthocyanin biosynthesis during fruit development in 'Nyoho' strawberry and found that fruit pigmentation in most of the strawberry cultivars have affect of amount of light falls on the fruit surface. But fruit colour was uniform in 'Nyoho' strawberry without any consideration of incident sunlight or shading. Matsui *et al* (2001 a, b) studied the effect of natural anthocyanins on the activity of alpha-glucosidase (AGH). They performed the alpha-glucosidase assay in 12 anthocyanin extracts which showed high AGH inhibitory activity. Stintzing *et al* (2002a) suggested that whole pigment extracts from blackberry being devoid of acetylated structures afforded higher antioxidant values than those from black carrot and red cabbage that contain a high percentage of acylated structures. Stintzing *et al* (2002b) found that pigments substituted with sugars both at the C3 and C5 position exhibited a lower antioxidant capacity than their corresponding anthocyanin-3-gycosides. Wang and Mazza (2002) firstly reported inhibitory effect of anthocyanins on nitric oxide production in numerous mammalian cells and tissues which is thought to associated with some chronic inflammatory disease. Nakajima *et al* (2003) found that introduction of an acyl moiety to anthocyanins and flavonols resulted into either retaining or decreasing the potential to scavenge the free radicals. OptiBerry is the health-promoting product in the form of a synergistic combination of six selected extracts from wild blueberry, bilberry, cranberries, elderberries, raspberries and strawberries. Lee *et al* (2005) determined the monomeric anthocyanins in fruit juices, beverages, natural colorants and wines by using AOAC official method and reported that anthocyanin content expressed as cyanidin-3-glucoside equivalents

ranged between 20 to 3000 mg/l. Zafra-Stone *et al* (2007) reported antioxidant and antiangiogenetic, anticarcinogenic and antibacterial properties of optiberry.

2.1.1 Anthocyanins as colouring agents

The anthocyanins are attractive for food colouring purposes because they show stability at increased pH. Acylated anthocyanins have been shown to be a promising alternative to synthetic colorants in food systems. Acylation of anthocyanins improves colour and pigment stability. The acetylated pelarogonidin derivatives from red radish could impart red colour to maraschino cherries extremely close to that of synthetic colorant FD&C Red#40 at pH 3.5 (Giusti and Wrolstad 1996). Bridle and Timberlake (1997) extracted anthocyanins from red cabbage and found that these were remained stable at broader pH range. Therefore, they suggested anthocyanins from red cabbage can be used as a natural alternative to synthetic blue colourings for foods with neutral pH. Further Rodriguez-Saona *et al* (1998) and Cevallos-Casals and Cisneros-Zevallos (2004) suggested that this synthetic colorant can be replaced by a natural one obtained from red potatoes (*Solanum tuberosum)*. Purple corn (Aoki *et al* 2002) and Oxalis (*Oxalis triangularis)* (Pazamino-Duran *et al* 2001) were also considered to be good sources of food colour.

2.1.2 Anthocyanins in Brinjal

Different kinds of anthocyanins have been extracted and identified from eggplant skin. Nasunin (delphinidin-3-p-coumaroylrutinosode-5-glucoside) is most commonly found, was first reported by Kuroda and Wada (1933, 1935). Pifferi and Zamorani (1969) and Matsuzoe *et al* (1999) studied six cultivars and eight cultivars/lines respectively and identified nasunin as a major anthocyanin present in eggplant skin (ranging from 69.1% to 87.7%). Tanchev *et al* (1970) identified delphidin-3-rutinoside and a smaller amount of delphidin-5-glucoside from the skin of Bulgarian eggplant. Guisti and Wrolstad (2005) reported anthocyanin content of 450.1 mg/kg fresh weight of eggplant as determined by pH differential method. Wu and Prior (2005) identified delphinidin-3-rutinoside as major anthocyanin in eggplant which was procured from US market. Similar to purple sweet potato, brinjal genotypes having purple coloured fruits consist of acylated anthocyanins which were considered to be more stable than non-acetylated anthocyanins (Ichiyanagi *et al* 2006). Wu *et al* (2006) reported that brinjal contain 85.70 mg anthocyanin content in 100g of fruit.

2.2 Enzymes

2.2.1 Phenylalanine ammonia lyase (PAL)

Phenylalanine ammonia lyase (PAL) (EC 4.3.1.24) is a key enzyme in the biosynthesis of phenolics in plants. It catalyses the non-oxidative deamination of phenylalanine to trans-cinnamate and ammonia (Koukol and Conn 1961, Bhattacharyya and Ward 1988) (Fig 3) and directs the carbon flow from the shikimate pathway to the branches of phenylpropanoid metabolism. Thus, catalytic step by PAL enzyme in plants is considered

to be the first committed step for biosynthesis of phenolics, flavonoids, phenylpropane and lignins (Cheng and Breen 1991). Aoki *et al* (1970) reported PAL activity only in the red sections of apple fruit skin and concluded that the activity of PAL was closely related to formation of anthocyanin. Camm and Towers (1973) reported that the level of PAL activity depends upon the genotype, age, development stage, organ and even tissue type of the plant. PAL is an important regulatory enzyme of secondary metabolism in plants and is regulated by various external and internal factors such as nutrient levels, light, fungal infection and wounding (Jones 1984). Jones (1984) demonstrated the concomitant increase in PAL activity and flavonoid compounds. Kuhn *et al* (1984) found that fungal infection results in increased synthesis of PAL which further results into synthesis of phenolic compounds. Increased expression of PAL enhances the accumulation of phenylpropanoids such as chlorogenic acid while major flavanoids such as rutin were not altered in tobacco plants which overexpress PAL (Bate *et al* 1994, Howles *et al* 1996, Shadle *et al* 2003). Dixon and Paiva (1995) found that PAL induces polypropanoid biosynthesis in response to biotic and abiotic stresses such as pathogen attacks, UV irradiation, mechanical wounding and light.

Yoshioka *et al* (1996) studied PAL activity in tubers and found that it increases in response to light, infection or treatment with elicitors. Weisshaar and Jenkins (1998) suggested that PAL may be responsible for many essential functions including establishing mechanical support, production of pigments such as anthocyanins and signalling with nodulation factors. Sharma *et al* (1999) found that exposure to UV-B lead to stimulation of PAL activity in rice, maize and turnip. PAL is considered to be marker of environmental stress in plant tissues. Sanchez-Ballesta *et al* (2000) reported the increase in activity of enzyme during exposure to low temperature. Kumar and Knowles (2003) found that after wounding or infection of the tissue, the PAL activity rose to levels of 150 times than normal. PAL activity and phenylpropanoids increased after wounding, during which a suberized wound periderm is formed. Butelli *et al* (2008) reported that in transgenic plant Del-Ros1 the increase in PAL transcript and enzymatic activity seemed to be important to obtain very high levels of anthocyanins. Zhang *et al* (2013) suggested that due to close relation between phenolics and disease resistance PAL is regarded as one of the important pathogenesis-related proteins.

2.2.2 Tyrosine ammonia lyase (TAL)

Tyrosine ammonia lyase (TAL EC 4.3.1.23) is an enzyme required for deamination of the amino acid L-Tyrosine to p-coumaric acid (Neish 1961) with the release of ammonia (Fig 3). Tyrosine ammonia-lyase (TAL) is a recently described member of the aromatic amino acid ammonia-lyase family and is one of the major enzymes involved in phenol biosynthesis pathway of plants whose activity is analogous to that of PAL. Koukal and Conn (1961) partially purified TAL and suggested that it is very labile enzyme.

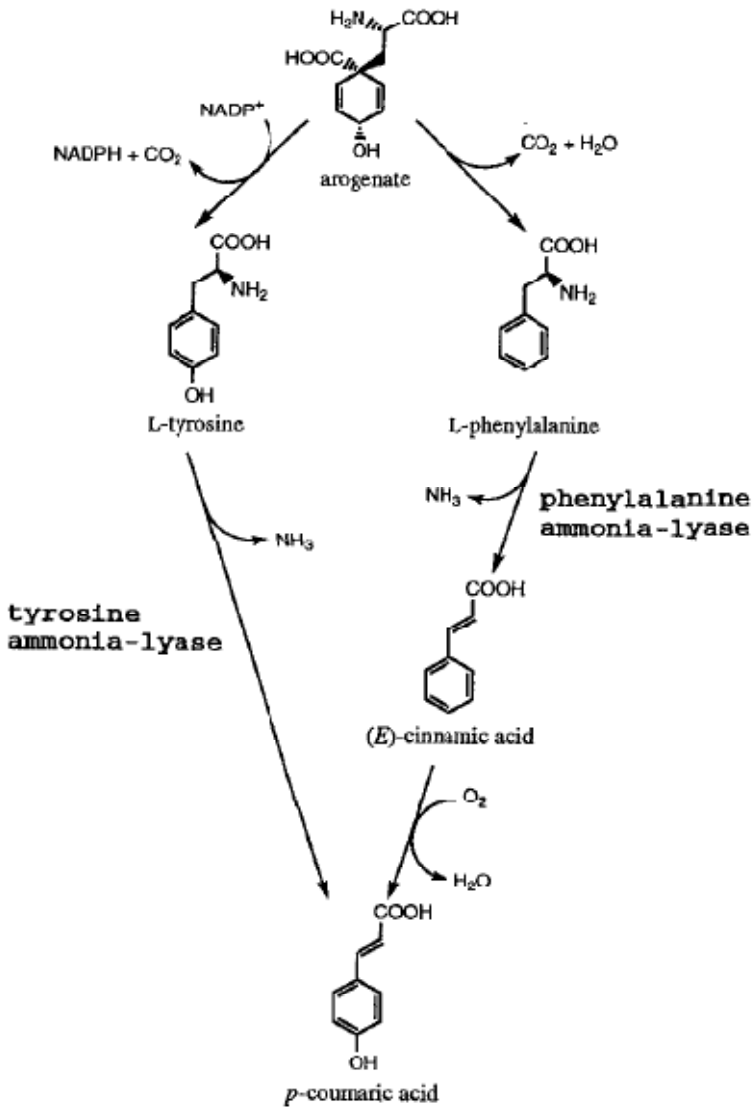


Fig 3: Reactions catalyzed by Phenylalanine ammonia lyase (PAL) and Tyrosine ammonia lyase (TAL).

The plant might lose TAL activity as it ages. Neish (1961) found no TAL in 30- to 40-day old alfaalfa plants, although young plants showed the enzymatic activity. Higuchi (1966) studied enzyme activity in bamboo in stem elongation zone and concluded that older tissue has low activity. It is predominately found in Gramineae, (Young *et al* 1966, Shah and Mehta 1978) also reported the same in dicotyledons. Morrison and Buxton (1993) proposed that products of PAL and TAL are modified through phenylpropanoid metabolism, produces precursors of secondary metabolites such as lignin, flavonoid pigments and phytoalexins. Rosler *et al* (1997) proposed that in monocots, the activities of PAL and TAL reside on the same polypeptides and both have similar catalytic efficiencies. It is found that TAL to be involved in the production of p-coumaric acid as the chromophore of the bacterial

photosensory yellow protein (Kort *et al* 1998, Kyndt *et al* 2002). Kyndt (2002) found that the TAL have 150 times less catalytic efficiency for L-phenylalanine than that for L-tyrosine and suggested that TAL mainly involved in the production of p-hydroxycinnamic acid. Nishiyama *et al* (2010) studied the TAL in *Arabidopsis thaliana* and found that expression of TAL enhanced the metabolic flux into the phenylpropanoid pathway which results into accumulation of phenylpropanoid derivatives such as quercetin glycosides.

2.2.3 Anthocyanidin synthase (ANS)

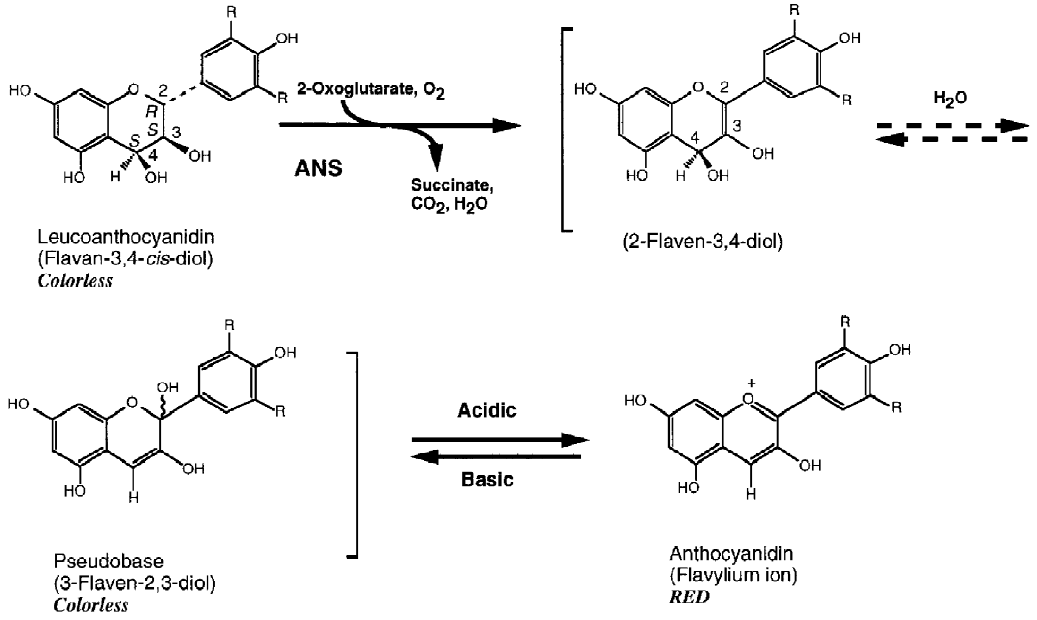


Fig 4: Conversion of leucoanthocyanidin to anthocyanidin by anthocyanidin synthase (ANS) (Saito *et al* 1999).

ANS is an important enzyme of anthocyanins biosynthesis pathway. It catalyzes the conversion of colourless leucoanthocyanidins to coloured anthocyanidins (shown in Fig 4). Anthocyanidins are extremely unstable and are converted to anthocyanins. Saito *et al* (1999) carried out first biochemical investigation of ANS by isolating recombinant form of enzyme from *Perrilla frutescens.* Anthocyanidin synthase (ANS) belongs to the 2-oxoglutarate iron-dependent oxygenases (Baldwin *et al* 1987, Akhtar and Wright 1991, Feig and Lippard 1994) which uses molecular oxygen and 2-oxogultatrate as co-substrates, Fe^{2+} as a cofactor and ascorbate as the reducing agent (Prescott and John 1996, Prescott *et al* 2002, Schofield and Zhang 1999). Pelletier *et al* (1997) and Gollop *et al* (2001) reported that the ANS expression is strongly associated with proanthocyanidin and anthocyanin accumulation. Saito *et al* (1999) suggested that ANS is an extremely unstable enzyme, this might be because of oxidation. Turnbull *et al* (2000) found that ANS requires high concentration of ascorbate and has less than 5% of full activity in the absence of ascorbate. Nakajima *et al* (2001) postulated

a mechanism that ANS catalyzed the step in which reaction proceeds from leucoanthocyanidin via 2-flaven-3, 4-diol (pseudobase) followed by isomerisation of the 2, 3-double bond to the 3, 4-position concomitant with a shift of the hydroxyl group from C-4 to C-2 and removal of the C-2 hydroxyl anion under acidic conditions to yield anthocyanidin (flavylium ion). It is also found that the synthesis of pure leucoanthocyanidin enantiomers is rather difficult and flavan-3, 4-diols in aqueous solution were unstable. It was also found that leucoanthocyanidin was a natural precursor of anthocyanidin as ANS is highly substrate specific. Turnbull *et al* (2003) studied that ANS forms quercetin as a major product with cyanidin being a minor product *in vitro.*

2.3 Other Phenolic Compounds

Phenolic compounds constitute one of the main classes of secondary metabolites. They display a large range of structures and they are responsible for characteristic colour and taste properties and also contribute to the nutritional qualities of fruits and vegetables. The family of secondary metabolites derived from aromatic metabolism include phenolics, coumarins, tannins, flavonoids, and polyphenols or phenylpropane derivatives. Phenolics have important roles in plants such as in defence against herbivores and pathogens, mechanical support, attracting pollinators and most importantly act as antioxidant compounds. Antimicrobial activities of polyphenols were studied by Tranter *et al* (1993) and Nachyas (1995) and they proposed them as potential natural preservatives. Hollman *et al* (1995) studied the absorption of qucercetin glycosides from onion on volunteers and they found that in healthy humans these were absorbed and eliminated slowly through the day (Hollman *et al* 1996). Free radical scavenging (antioxidant) activity of phenolic compounds was determined by their reactivity as hydrogen- or electron donating agents, the stability of the resulting antioxidant-derived radical, their reactivity with other antioxidants and their metal chelation properties (Rice-Evans *et al* 1996). Plasma antioxidant activity was reported to increase after consumption of red wine (Cao *et al* 1998, Serafini *et al* 1998, Whitehead *et al* 1995) and grape wine (Day *et al* 1997). Gee *et al* (1998) proposed that the quercetin glucosides are capable of interacting with glucose transporter receptors in the mucosal epithelium and, therefore, may be absorbed by the small intestine *in vivo.* Vinson *et al* (1998) determined that vegetables have antioxidant quality comparable to that of pure phenols and superior to that of the antioxidant vitamins A, C and E. Curir *et al* (2001, 2003) and Galeotti *et al* (2008) proposed that phenolic compounds such as flavonoids may act as phytoanticipins. Cacace and Mazza (2002) extracted the phenolics from black currant using different SO_2 concentrations and solvents, while effect of pH and temperature on anthocyanin extraction from black carrots was studied by Türker and Erdoğdu (2006). Potatoes with high amounts of phenylpropanoids were reported to reduce inflammation in human feeding studies (Kaspar *et al* 2011) and immature potatoes were found to be responsible for lower blood pressure and to

raise serum antioxidants (Vinson *et al* 2012).

2.3.1 Phenolic Compounds of Brinjal

Out of 120 vegetables evaluated for antioxidant activity, brinjal ranked among the top ten for superoxide scavenging activity. Winter and Herrmann (1986) proposed that esters of hydroxycinnamic acids (HCAs) are the major class of phenolic compounds in brinjal fruit, in which chlorogenic acid is predominant. Sudheesh *et al* (1997) studied hypolipidemic beneficial effect of phenolic compounds extracted from eggplant fruit in normal and cholesterol fed rats. Stommel and Whitaker (2003) also studied the diversity of phenolic compounds found in eggplant. Tomas-Barberan *et al* (2001) and Jhang *et al* (2010) proposed that eggplant peel contains higher amounts of phenolics, anthocyanins and flavonols than pulp tissue. Huang *et al* (2004) reported antioxidant activity of eggplant and found that crude eggplant extract can inhibit low density lipids oxidation. Lutharia and Mukhopadhyay (2006) optimized extraction of phenolic acids from eggplant. Sadilova *et al* (2006) reported that the flavonols isolated from eggplant exhibit antioxidant activity against chromosomal aberrations induced by doxorubicin. Singh *et al* (2009) reported the relationship between phenolic content and antioxidant activity of eggplant pulp. Jhang *et al* (2010) showed that eggplant calyx exhibited five times higher amounts of total phenolic content than the flesh. Due to higher content of phenolic compounds calyx part of plant have been used as a traditional medicine (Bruni *et al* 2004, Hirunpanich *et al* 2006).

CHAPTER III

MATERIAL AND METHODS

Procurement of samples

The present investigation, "A study on anthocyanins and other biochemical constituents in different plant parts of brinjal" was carried out in the field and biochemistry laboratory of Department of Vegetable Science, Punjab Agricultural University, Ludhiana. The material and methods employed in the present study are described under the following headings:

3.1 Raising of crop and procurement of Samples

Crop of brinjal was raised at the Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana.

3.2 Collection of Samples

The fruit and leaf samples were collected at 7^{th} day after anthesis (DAA), the subsequent second and third samples were taken at an interval of 7 days (i.e 14 and 21 DAA). For enzyme assay, various extracts were prepared and the activity of phenylalanine ammonia lyase, tyrosine ammonia lyase and anthocyanidin synthase was determined in these extracts. The brinjal fruit samples were harvested at maturity and analyzed for agronomic characters

and biochemical parameters such as anthocyanins, dry matter, total phenols, ortho-dihydroxy phenols and flavonols.

3.3 Extraction and assay of Phenylalanine ammonia lyase (PAL) (Hadwiger and Schwochau, 1971)

Principle

PAL catalyzes the deamination of L-phenylalanine to yield trans-cinnamic acid which is the first step in the biosynthesis of phenylpropanoids, which are further modified into a wide variety of phenolic compounds.

A) Extraction

a) Reagents

i) Extraction Buffer: 0.5M Potassium phosphate buffer (pH 8.0)

ii) 0.5g PVP

b) Extraction

Five hundred milligram of fresh sample was homogenized in a chilled mortar with 5 ml 0.5M potassium phosphate buffer (pH 8.0) in the presence of polyvinylpyrrolidone (PVP). This and subsequent extractive operations were carried out at 4˚C. The homogenate was centrifuged at 20,000g for 10 min at 4˚C. The supernatant was used for PAL assay.

B) Assay

a) Reagents

i) Working buffer (0.05 M Boric acid-borax buffer (pH 9.0): 50 ml of 0.05M boric acid and 59 ml of 0.05M borax crystals solution were mixed and diluted to 200 ml.

ii) 10mM L-phenylalanine: 0.17g of L-phenylalanine was dissolved in 100 ml of distilled water.

iii) 5N HCl: 41 ml of HCl was dissolved and volume was made to 100 ml with distilled water.

iv) 0.05M NaOH: 0.2g of NaOH was dissolved volume was made to 100 ml with distilled water.

v) Diethyl ether

b) Procedure

The assay mixture contained 0.1 ml enzyme extract, 0.5 ml 10mM L-phenylalanine and 3.0 ml 0.05 M Boric acid-borax buffer (pH 9.0). The reaction mixture was incubated for 1 h at 37˚C. The reaction was then stopped by adding 0.1 ml of 5N HCl. The acidified mixture was extracted twice with 7.5 ml diethyl ether. The ether extracts were pooled and evaporated to dryness under a stream of air. The residue was dissolved in 5ml 0.05M NaOH. The cinnamic acid formed at the end of the reaction was estimated by measuring the absorbance at 290 nm in a LABINDIA 3000^{+} UV/VIS Spectrophotometer. Standard curve was prepared by using trans-cinnamic acid in the concentration range 5-35µg/ml.

3.4 Tyrosine ammonia lyase (TAL) (Burrell and Rees 1974)

Principle

Tyrosine ammonia lyase is an enzyme required for deamination of amino acid L-tyrosine to p-coumaric acid. It is the one of the major enzymes involved in phenol biosynthesis pathway of plants.

A) Extraction

a) Reagents

i) **Extraction Buffer:** 0.5M Potassium phosphate buffer (pH 8.0)

ii) **0.5g PVP**

b) Extraction

Five hundred milligram of fresh sample was homogenized in a chilled mortar with 5ml 0.5M potassium phosphate buffer (pH 8.0) in the presence of polyvinylpyrrolidone (PVP). This and subsequent extractive operations were carried out at 4˚C. The homogenate was centrifuged at 20,000g for 10 min at 4˚C. The supernatant was used for TAL assay.

B) Assay

a) Reagents

i) **Working buffer (0.05M Boric acid-borax buffer (pH 9.0)):** 50 ml of 0.05M boric acid and 59 ml of 0.05M borax crystals solution were mixed and diluted to 200 ml.

ii) **33µM tyrosine:** 0.59 g of tyrosine dissolved and volume was made to 100 ml with distilled water.

iii) **5N HCl:** 41 ml of HCl was dissolved and volume was made to 100 ml with distilled water.

b) Procedure

The assay mixture contained 0.2 ml enzyme extract, 1.0 ml 33µM tyrosine and 4.8 ml 0.05M boric acid-borax buffer (pH 9.0). The reaction mixture was incubated for 1 h at 37˚C. The reaction was then stopped by adding 0.1 ml 5N HCl. The p-coumaric acid formed at the end of the reaction was estimated by measuring the absorbance at 310 nm in a LABINDIA 3000^{+} UV/VIS Spectrophotometer. Standard curve was prepared by using p-coumaric acid in the concentration range 5-35µg/ml.

3.5 Anthocyanidin Synthase (ANS) (Saito *et al* 1999)

Principle

It is an enzyme of anthocyanins biosynthetic pathway, catalyzes the conversion of colourless leucoanthocyanidins to the coloured anthocyanins.

A) Extraction

a) Reagents

i) 0.5M Potassium phosphate buffer (pH 7.0): 5.3 ml of 0.5M monobasic potassium phosphate and 94.7 ml of 0.5M dibasic potassium phosphate were mixed and diluted to

200 ml.

ii) 0.5g PVP

b) Extraction

Five hundred milligram of fresh sample was homogenized in a chilled mortar with 5ml of 20mM potassium phosphate buffer (pH 7.0) in the polyvinylpyrrolidone (PVP). This and subsequent extractive operations were carried out at 4˚C. The homogenate was centrifuged at 40,000g for 10 min at 4˚C.The supernatant was used for ANS assay.

B) Assay

a) Reagents

i) 20mM potassium phosphate buffer (pH 7.0) containing 200mM NaCl, 10mM maltose, 5mM sodium ascorbate, 1mM 2-oxoglutaric acid and 0.4mM $FeSO_4$.

ii) 1mM trans-dihydroquercetin

b) Procedure

The assay mixture contained 0.1 ml enzyme extract, 20mM potassium phosphate buffer (pH 7.0), 200 mM NaCl, 10 mM maltose, 5mM sodium ascorbate, 1mM 2-oxoglutatric acid and 0.4mM $FeSO_4$.The reaction was initiated by the addition of 0.5 ml 1mM trans-dihydroquercetin (DHQ). The conversion of DHQ to quercetin by ANS was monitored for 2 min in a LABINDIA 3000 $^+$ UV/VIS Spectrophotometer at 30˚C. The enzymatic activity was represented as a change in the absorbance at 360 nm due to the conversion of DHQ to quercetin.

3.6 Estimation of Proteins in Enzyme Extract (Lowry *et al* 1951)

Principle

This method utilizes Folin-phenol reagent. The protein forms a blue coloured complex with ions in alkaline solution. The intensity of colour is measured at 520 nm. The protein concentration is determined with Folin-Ciocalteau's phenol reagent, which couples redox reaction (Cu^+ ions formation, biuret method) with a second redox reaction (colour change).

a) Reagents

i) Reagent A: 2% sodium carbonate in 0.1N sodium hydroxide.

ii) Reagent B: 0.5% cupric sulphate in 1% sodium potassium tartarate.

iii) Reagent C: Alkaline copper tartarate. It was freshly prepared by mixing 50 ml of reagent A with 1 ml of reagent B just before use.

iv) 1N Folin-Ciocalteau reagent.

Procedure

To 0.1 ml of enzyme extract, 0.9 ml of distilled water and 5 ml of reagent C was added. Shaken thoroughly and kept for 10 min. Then added 0.5 ml of Folin-Ciocalteau reagent and again shaken thoroughly. After keeping for 30 min, blue colour developed was

read at 520 nm against the reagent blank. Bovine serum albumin (BSA) was used as standard in the range 10-100 µg/ ml. The concentration of protein was calculated from standard curve.

3.7 Extraction and estimation of biochemical quality parameters in brinjal

3.7.1 Extraction and estimation of Anthocyanins (Rabino *et al* 1977)

Fruit samples were crushed and anthocyanins extracted with 10 ml of 1% HCl (w/v) in methanol for overnight at 4˚C. The absorbance of extracts which were clarified by filtration was measured at 530 nm and 657 nm with a LABINDIA 3000^+ UV/VIS Spectrophotometer. The anthocyanin content of the extracts was presented as A_{530}-$0.33A_{657}$. This formula is used to correct for the contribution of Chl and its degradation products in acid solution to the absorbance of the extracts at 530 nm.

3.7.2 Dry Matter (AOAC 1970)

Fifty gram of sliced fruit of each sample was dried in a pre-weighed petri-plates at 65±2˚C till constant weight was obtained. The dried samples were cooled in a desiccator for 10 minutes. These were weighed and the dry matter percentage was then calculated by following formula:

$$\text{Dry Matter Content (\%)} = \frac{\text{Final dry weight of the sample}}{\text{Initial fresh weight of the sample}} \text{ X } 100$$

3.7.3 Extraction and estimation of Total Soluble Sugars (Dubois *et al* 1956)

Principle

Sugars form furfurals and hydroxyl-methyl furfurals (pentoses and hexoses respectively) in the presence of conc. H_2SO_4, which react with phenol to give coloured compounds. All sugars give this test as oligo and polysaccharides will get hydrolyzed with conc. H_2SO_4 and also form furfurals.

A) Extraction procedure

a) Reagents

i) Ethanol (80%)

ii) Lead acetate (saturated)

iii) Sodium oxalate

Weighed 500 mg dried samples and refluxed with 80% ethanol in a conical flask fitted with water condenser and centrifuged at 5000 rpm for 15 min. Supernatant was collected, the residue left over was given twice washings with 80% aqueous ethanol and the process was repeated. Supernatants were collected and pooled. Ethanol from pooled extract was removed at 50˚C in a flash evaporator under vaccum. Then 1.0 ml of saturated lead acetate was added and volume of the extract made to 100 ml with distilled water. This was kept overnight till all proteins in the extract get precipitated. To this a pinch of sodium oxalate

was added to remove lead ions from extract. It was again kept overnight. Thereafter, the extract was filtered through Whatmann no. 1 filter paper. The clear extract thus obtained was used for sugar estimation.

B) Estimation

a) Reagents

i) 5% phenol (w/v) redistilled

ii) Conc. H_2SO_4

b) Procedure

To 0.1 ml of sugar extract, 0.9 ml of distilled water and 1.0 ml of phenol was added followed by the addition of 5.0 ml of conc. H_2SO_4. The H_2SO_4 was poured directly in the centre of the test tube to ensure proper mixing. After 10 min tubes were cooled to room temperature with running water. After 20 min the absorbance was read at 490 nm against reagent blank. The concentration of total sugars was read from standard curve prepared by using glucose in the range of 10-80 µg/ml.

3.7.4 Extraction and estimation of Phenolic Compound

Extraction

Weighed 500 mg dried fruit samples and refluxed with 80% methanol for 2 h. The refluxed material was filtered and volume was made 25 ml by washing with 80% methanol. The extract thus prepared was used for estimation of phenolic content viz. total phenols, ortho-dihydroxyphenols and flavonols.

3.7.4.1 Estimation of total phenols (Swain and Hillis, 1959)

A) Reagents

i) Folin-Ciocalteu reagent: 2N Folin-ciocalteu reagent diluted 1:1 (v/v) with distilled water

ii) Saturated solution of Na_2CO_3: 17.5g of Na_2CO_3 was dissolved in 50 ml of distilled water.

B) Procedure

Methanolic extract (5 ml) was evaporated to dryness and the residue was dissolved in 6.5 ml of distilled water. To this 0.5 ml of Folin-Ciocalteu reagent was added and shaken thoroughly. After 3 min, 1 ml of saturated solution of Na_2CO_3 and volume was made 25 ml with distilled water. After 1 h, the absorbance of blue colour was read in a spectrophotometer at 760 nm against the blank. The blank was prepared from water and reagents only. The concentration of total phenols was determined from standard curve prepared by using catechol (20-100µg/ml).

3.1.4.2 Estimation of ortho-dihydroxy phenols (Nair and Vaidyanathan, 1964)

A) Reagents

i) 10% Trichloroacetic acid (TCA): 10g of TCA was dissolved in 100 ml of water.

ii) 10% Sodium tungstate: 10 g of sodium tungstate was dissolved in 100 ml of water.

iii) 0.5 N Hydrochloric acid (HCl): 2.5 ml of HCl was diluted in 50 ml of water.

iv) 0.5 % Sodium nitrite: 0.5 g of sodium nitrite was dissolved in 100 ml of water.

v) 0.5 N Sodium hydroxide: 0.2 g of sodium hydroxide was dissolved in 100 ml of water.

B) Procedure

Methanolic extract (5.0 ml) was evaporated to dryness and residue left behind was dissolved in 1.0 ml of distilled water. To this 0.5 ml of 10% TCA, 1.0 ml of sodium tungstate, 0.5 ml of 0.5 N HCl, and 1.0 ml of freshly prepared 0.5% sodium nitrite were added. A yellow colour developed. After 5 min 2.0 ml of 0.5 N sodium hydroxide was added. The light cherry colour developed, whose absorbance was read after 15 min at 540 nm against the blank. The blank consisted of water and reagents only. The concentration of ortho-dihyroxyphenols was determined from standard curve prepared by using catechol (10-100µg/ml).

3.1.7.3 Estimation of Flavonols (Balabaa *et al* 1974)

A) Reagents

0.01 M methanolic solution of aluminium chloride ($AlCl_3$).

B) Procedure

5.0 ml of methanolic extract was evaporated to dryness. The residue left was dissolved in 10 ml of 0.1 M methanolic solution of aluminium chloride. Yellow colour developed, which was read at 420 nm against 0.1 M methanolic solution of $AlCl_3$ as blank. The concentration of flavonols was determined from standard curve prepared by using rutin (50-250 µg/ml).

3.8 Agronomic Characters

3.8.1 Fruit Length

Length of three fruits of a sample was measured in centimetres.

3.8.2 Fruit Width

Width of three fruits of a sample was measured in centimetres.

3.8.3 Fruit Weight

Weight of three fruits of a sample was measured in grams.

3.9 Statistical analysis

Statistical analysis was performed in triplicate for each physical and biochemical parameter as well as enzymes. Data are presented as mean±SD. The variance analysis and significant differences among the means were analyzed with two-way analysis of variance (ANOVA) by using CPCS software version 1.0. The correlation among various biochemical parameters was shown by using pearson coefficient of correlation.

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation was conducted to select the brinjal genotypes for high anthocyanin content and other biochemical parameters such as dry matter, total soluble sugars, total phenols, ortho-dihydroxy phenols and flavonols and also to study the activities of enzymes in selected genotypes.

The results of present investigation have been presented and discussed under the following sub headings:

4.1 Screening of brinjal genotypes for anthocyanin content along with other parameters such as dry matter, total soluble sugars, total phenols, ortho-dihydroxy phenols and flavonols.

4.2 Enzymatic studies on selected genotypes for anthocyanin synthesizing enzymes in leaves and fruits at various stages of fruit ripening.

4.1 SCREENING OF BRINJAL GENOTYPES FOR ANTHOCYANIN CONTENT ALONG WITH OTHER PARAMETERS SUCH AS DRY MATTER, TOTAL SOLUBLE SUGARS, TOTAL PHENOLS, ORTHO-DIHYDROXY PHENOLS (ODHs) AND FLAVONOLS

4.1.1 Anthocyanin content

Anthocyanins are naturally occurring pigments present in peel and to a lesser extent, in flesh of brinjal fruit and give purple and/or red colour to the fruit (Mazza *et al* 2004). Anthocyanins are widely studied for their medical potential (Kong *et al* 2003). The present study was conducted to analyse the amount of anthocyanins present in various genotypes of brinjal. In present study anthocyanins were extracted from peel, flesh part and whole fruit of various brinjal genotypes. 50 genotypes of brinjal were selected for the analysis of anthocyanins content. The genotypes of brinjal under study were having different colours namely purple, dark purple, pink, purple with green streaks, green, green with white streaks and pure white. Anthocyanins extraction from fresh fruit was done in acidified methanol which can yield 80% of total content (Metivier *et al* 1980).

Variation in anthocyanin content in brinjal genotypes under investigation is reported for two years 2012 and 2013. Genotypes having purple fruits have the highest content followed by genotypes having pink fruits. Brinjal genotypes having white and green fruits have very low or negligible anthocyanin content in peel, even sometimes it could not be detected. The anthocyanin content was found in the range of 0.04-113.93 mg/100g in 2012 and 0.05-109.02 mg/100g in 2013 (Table 1). The average anthocyanin content in 2012 was 58.89 mg/100g with the highest content 113.81±1.9 mg/100g in SR-312. In 2013, the average anthocyanin content was found 59.12 mg/100g with the highest content 107.70±1.3 mg/100g in SR-312. Anthocyanin content within two years was statistically not significant. Jung *et al*

Table 1: Anthocyanin content in peel of different genotypes in 2012 and 2013.

Name of the Genotype	Colour of the genotype	Anthocyanin content (mg/100g)		Mean
		2012	2013	
BL-2001-1-2	White	ND	ND	**ND**
BL-201	Purple	86.78±0.3	99.83±0.4	**93.30**
BL-202	Purple	99.95±0.1	98.73±0.0	**99.34**
BL-204	Purple	93.33±0.1	99.97±0.5	**96.65**
BL-207	Purple	94.23±2.8	99.95±1.4	**97.09**
BL-214	Purple	96.44±0.0	105.11±0.0	**100.77**
BL-215	Purple	105.14±0.3	100.19±0.3	**102.66**
BL-219	Purple	98.39±1.8	104.15±1.4	**101.27**
BL-220	Purple	98.56±0.3	103.07±0.2	**100.81**
BLW-231	White	0.07±0.1	0.05±0.0	**0.06**
BLEND-11-WR-1	White	3.34±0.6	3.10±0.5	**3.22**
BLEND-11-WR-2	White, purple strips	0.04±0.0	0.18±0.1	**0.11**
SR-308	Green	3.16±0.6	3.13±0.7	**3.14**
BR-104	Purple	93.01±0.2	99.30±0.4	**96.15**
BR-118	Purple	104.01±0.4	100.48±0.6	**102.25**
BR-133	Purple	92.49±0.0	87.89±25.1	**90.18**
BRG-111	Pink	57.41±0.1	62.00±0.0	**59.70**
BRG-224	Pink	74.11±10.6	69.65±10.4	**71.88**
G-401	Green	ND	ND	**ND**
G-402	Green	1.25±0.2	ND	**0.63**
G-403	Green	7.09±0.6	4.60±0.7	**5.84**
G-405	Green	5.64±0.1	5.98±0.1	**5.81**
G-407	Green	4.24±0.2	1.98±0.3	**3.11**
G-408	Green	5.48±1.1	ND	**2.74**
G-409	Green	ND	ND	**ND**

G-411	Green	46.34±1.3	45.24±1.0	**45.79**
G-412	Green	ND	ND	**ND**
G-414	Green	ND	0.32±0.0	**0.16**
G-415	Green	ND	ND	**ND**
G-418	Green	ND	ND	**ND**
MR-319	Purple	106.67±2.2	103.49±1.4	**105.08**
MR-320	Purple	98.15±0.2	86.59±0.8	**92.37**
P-71	Purple	103.31±0.2	104.21±0.2	**103.76**
SR-301	Purple	104.21±1.8	106.06±1.6	**105.14**
SR-302	Purple	103.17±1.7	105.19±1.4	**104.18**
SR-303	Purple	104.00±1.4	103.33±0.9	**103.67**
SR-304	Purple	100.25±1.1	105.80±0.7	**103.03**
SR-305	Purple	97.83±0.2	99.84±0.8	**98.83**
SR-306	Purple	94.23±1.0	105.29±0.8	**99.76**
SR-307	Purple	105.17±2.4	104.90±2.9	**105.03**
SR-309	Purple	110.32±0.5	109.02±0.9	**109.67**
SR-310	Purple	107.56±2.8	103.97±2.5	**105.76**
SR-311	Purple	105.31±1.3	104.46±2.0	**104.88**
SR-312	Purple	113.81±1.9	107.70±1.3	**110.75**
SR-313	Purple	103.93±1.1	101.15±1.2	**102.54**
SR-318	Purple	112.19±0.7	105.62±0.8	**108.90**
SRV-360-1	White	ND	ND	**ND**
W-230-42-45-1	White	2.77±0.7	3.01±0.6	**2.89**
WL-502	White	0.59±0.3	0.39±0.2	**0.49**
WO-406	White	0.39±0.1	0.96±0.2	**0.67**
	MEAN	**58.89**	**59.12**	
CD (5 %)	A = Genotypes = 3.52 B = Anthocyanin content = NS AxB = 4.98			

ND = Not Detected, NS = Non-Significant

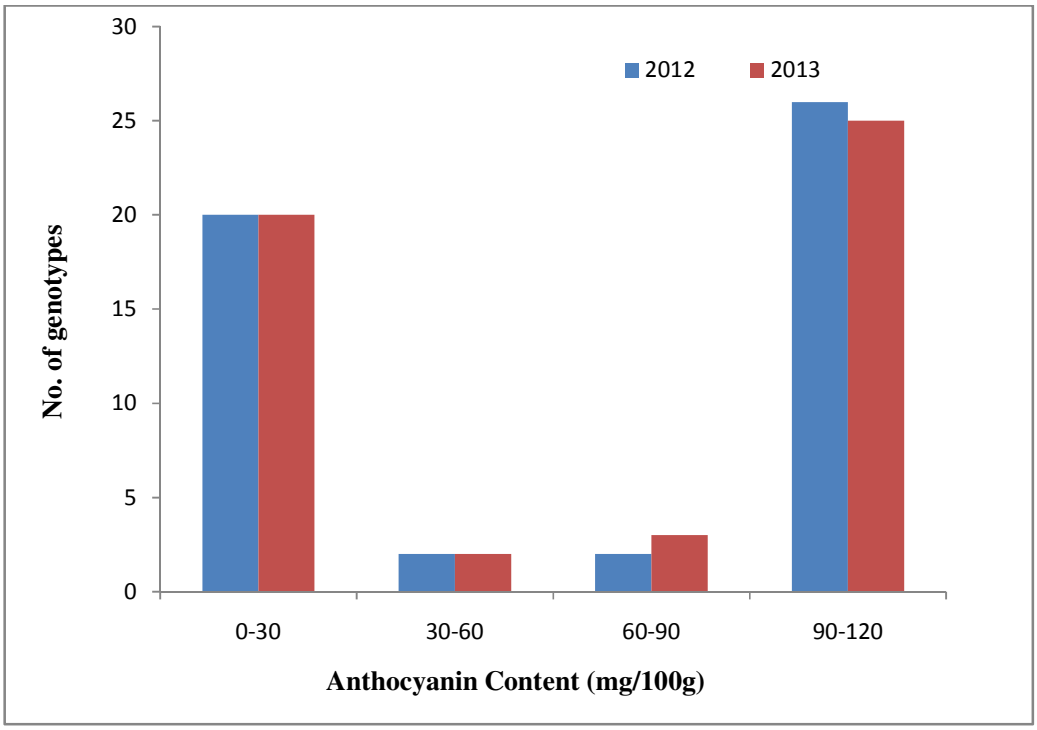


Fig 1: Frequency histogram showing distribution of anthocyanins (mg/100g) in peel of various genotypes of brinjal in 2012 and 2013.

(2011) found that the highest anthocyanin content in peel extract was 138.09 mg %. The highest anthocyanin content was found 110.75 mg/100g in genotype SR-312 in both years. The anthocyanins were not detected in BL-2001-1-2, G-401, G-409, G-412, G-415 and G-418 in two years. Frequency histogram showed the number of genotypes falling in different ranges (Fig 1).

In 2012, the maximum number of genotypes (26) showed anthocyanin content in range of 90-120 mg/100g which was maximum range. In 2013, the maximum number of genotypes (25) have anthocyanin content in maximum range of 90-120 mg/100g in peel. Table 2 shows elite genotypes having anthocyanin content in the maximum range of 90-120 mg/100g FW. This data confirmed that brinjal genotypes have high anthocyanin content in peel of purple fruits. The deep purple colour appearance has been reported to be due to the presence of delphidin-type pigments which are modified by acylation and total anthocyanins concentration (Sadilova *et al* 2006). Another factor responsible for colour was reported by Nothmann *et al* (1976) who suggested that the presence of chlorophylls could contribute to darkening.

Table 3 represents the anthocyanin content present in flesh of various brinjal genotypes. The anthocyanin content was found in the range of 0.01-9.89 mg/100g in 2012 and in range of 0.03-6.84 mg/100g in 2013. The average anthocyanin content was 1.02 mg/100g with the highest content 9.89±0.5 mg/100g in SR-308. In 2013, the average anthocyanin

Table 2: Elite genotypes of brinjal showing anthocyanin content > 90 mg/100g FW in 2012 and 2013.

Genotypes in 2012	Genotypes in 2013
BL-202	BL-201
BL-204	BL-202
BL-207	BL-204
BL-214	BL-207
BL-215	BL-214
BL-219	BL-215
BL-220	BL-219
BR-104	BL-220
BR-118	BR-104
BR-133	BR-118
MR-319	MR-319
MR-320	P-71
P-71	SR-301
SR-301	SR-302
SR-302	SR-303
SR-303	SR-304
SR-304	SR-305
SR-305	SR-306
SR-306	SR-307
SR-307	SR-309
SR-309	SR-310
SR-310	SR-311
SR-311	SR-312
SR-312	SR-313
SR-313	SR-318
SR-318	

Table 3: Anthocyanins content in flesh of brinjal genotypes in 2012 and 2013.

Name of the Genotype	Colour of the genotype	Anthocyanin content (mg/100g)		Mean
		2012	2013	
BL-2001-1-2	White	0.14±0.2	0.29±0.1	**0.21**
BL-201	Purple	0.01±0.1	0.67±0.1	**0.34**
BL-202	Purple	0.02±0.1	0.03±0.1	**0.03**
BL-204	Purple	0.03±0.2	0.46±0.4	**0.25**
BL-207	Purple	0.26±0.4	0.79±0.5	**0.52**
BL-214	Purple	0.65±1.1	ND	**0.33**
BL-215	Purple	0.02±0.0	0.36±0.1	**0.19**
BL-219	Purple	0.02±0.0	ND	**0.01**
BL-220	Purple	0.66±1.1	2.21±0.3	**1.44**
BLW-231	White	0.32±0.5	0.75±0.1	**0.54**
BLEND-11-WR-1	White	0.69±1.1	2.24±0.3	**1.46**
BLEND-11-WR-2	White with purple streaks	0.19±0.3	0.41±0.0	**0.30**
SR-308	Green	9.88±0.5	6.84±0.8	**8.36**
BR-104	Purple	0.23±0.3	0.13±0.1	**0.18**
BR-118	Purple	0.02±0.0	1.38±0.4	**0.70**
BR-133	Purple	0.18±0.1	0.24±0.1	**0.21**
BRG-111	Pink	ND	0.06±0.1	**0.03**
BRG-224	Pink	2.70±0.8	1.85±0.4	**1.96**
G-401	Green	0.13±0.1	ND	**0.07**
G-402	Green	0.39±0.5	0.12±0.1	**0.26**
G-403	Green	1.33±0.3	0.51±0.0	**0.92**
G-405	Green	1.44±0.4	1.70±0.1	**1.57**
G-407	Green	0.26±0.2	0.38±0.1	**0.32**
G-408	Green	0.28±0.2	0.18±0.1	**0.23**
G-409	Green	ND	ND	**ND**

G-411	Green	1.15±0.1	0.82±0.4	**0.98**
G-412	Green	0.27±0.2	0.88±0.1	**0.58**
G-414	Green	1.05±0.1	0.86±0.1	**0.95**
G-415	Green	1.17±0.2	1.11±0.0	**1.14**
G-418	Green	0.08±0.1	0.15±0.1	**0.12**
MR-319	Purple	1.42±0.6	1.73±0.1	**1.57**
MR-320	Purple	1.64±1.0	2.06±0.2	**1.85**
P-71	Purple	0.08±0.1	0.11±0.1	**0.10**
SR-301	Purple	1.52±0.4	1.65±0.2	**1.59**
SR-302	Purple	0.01±0.0	1.15±0.0	**0.58**
SR-303	Purple	1.38±0.3	1.17±0.3	**1.27**
SR-304	Purple	1.45±0.4	2.51±0.1	**1.98**
SR-305	Purple	0.44±0.3	1.59±0.2	**1.01**
SR-306	Purple	3.86±0.5	3.71±0.7	**3.78**
SR-307	Purple	0.30±0.2	0.38±0.0	**0.34**
SR-309	Purple	1.37±1.2	1.76±0.2	**1.56**
SR-310	Purple	1.72±1.1	1.97±0.3	**1.84**
SR-311	Purple	1.19±0.3	1.18±0.4	**1.18**
SR-312	Purple	2.17±0.2	1.63±0.4	**1.90**
SR-313	Purple	0.90±0.2	0.72±0.3	**0.81**
SR-318	Purple	3.55±0.6	2.93±0.8	**3.24**
SRV-360-1	White	1.04±0.1	0.96±0.1	**1.00**
W-230-42-45-1	White	1.22±0.2	1.23±0.3	**1.22**
WL-502	White	1.35±0.3	1.20±0.1	**1.27**
WO-406	White	0.99±0.1	1.19±0.2	**1.09**
	MEAN	**1.02**	**1.13**	
CD (5 %)	A = Genotypes = 0.44 B = Anthocyanin content = 0.09 AxB = 0.63			

ND = Not Detected

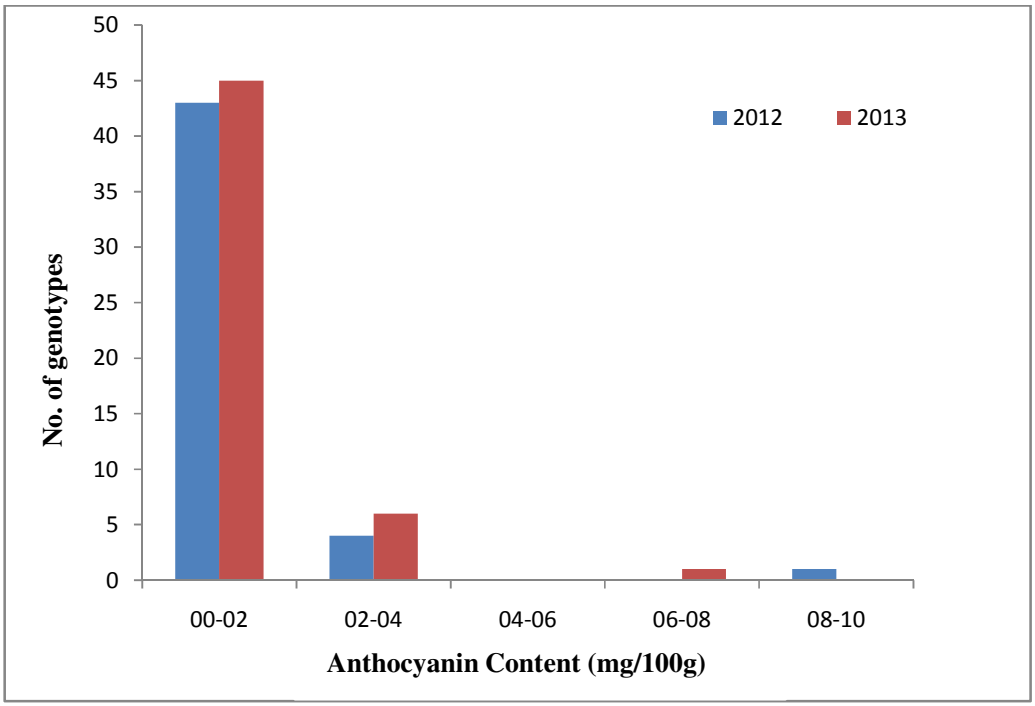


Fig 2: Frequency histogram showing distribution of anthocyanins (mg/100g) in flesh of various genotypes of brinjal in 2012 and 2013.

content was found 1.13 mg/100g with the highest content 6.84±0.8 mg/100g in SR-308. In both years, SR-308, genotype having green fruits has the highest anthocyanin content. Fig 2 shows the distribution pattern of anthocyanins in flesh part of brinjal genotypes. There were 43 genotypes having anthocyanin content in the range of 0-2 mg/100g in 2012 while 45 genotypes out of 50 have anthocyanin content in the same range in 2013. No genotype showed anthocyanin content in the range of 4-6 mg/100g. Only one genotype showed content in range of 6-8 mg/100g in 2013 and in the range of 8-10 mg/100g in 2012. Jung *et al* (2011) found that the highest anthocyanin content in flesh extract was 2.29 mg %. Table 4 shows brinjal genotypes having anthocyanin content > 2 mg/100g in flesh in 2012 and 2013.

Table 4: Elite genotypes of brinjal having anthocyanin content > 2 mg/100g in flesh in 2012 and 2013.

Genotypes in 2012	Genotypes in 2013
SR-308	SR-308
BRG-224	SR-304
SR-306	SR-306
SR-312	SR-312
SR-318	SR-318
	BLEND-11-WR-1

Anthocyanin content in whole fruit of various brinjal genotypes is represented by Table 5. In 2012, the whole fruit contained anthocyanins in the range of 0.55-88.24 mg/100g while in 2013, the content was found in the range of 1.87-88.91 mg/100g. The average content of total anthocyanins was 18.93 mg/100g in 2012 with the highest content of 88.24±4.6 mg/100g in SR-303. In year 2013, the average content of anthocyanins was 18.25 mg/100g. Similar to that in 2012, the highest anthocyanin content was found in genotype SR-303 (88.91±4.6 mg/100g) which was in accordance with the results shown by Wu *et al* (2006). Distribution of anthocyanins in whole fruit of brinjal is shown by Fig 3. Maximum number of genotypes (34 in 2012 and 35 in 2013) showed anthocyanin content in whole fruit in the range of 0-20 mg/100g followed by the genotypes (8 in both years i.e. 2012 and 2013) in the range of 20-40 mg/100g in both years. In two years only one genotype showed the anthocyanin content in whole fruit in the maximum range. Table 6 shows elite genotypes having anthocyanin content >60 mg/100g in whole fruit of brinjal genotypes in 2012 and 2013. These include genotypes having purple coloured fruits.

On comparing the average content of anthocyanins in peel, flesh and whole fruit (Table 7, Fig 4), it was found that peel contained the highest amount of anthocyanins followed by whole fruit and flesh part of brinjal. These results were in accordance with Jung *et al* (2011) who reported the highest anthocyanins content (138.05 mg %) in peel extract followed by calyx (135.94 mg %), stem (110.94 mg %), leaf (97.29 mg %) and pulp (2.29 mg %) extracts. There was not much difference in average anthocyanin content in two years in each plant part. However, there was considerable difference in anthocyanin content within same genotype with year difference in each plant part. Similar results are shown by Connor *et al* (2005) who showed the variation in anthocyanin content in blackberry and hybridberry in 2003 and 2005 and concluded that variations were due to interaction between cultivar and environment.

Brinjal is known to have low sugar content and thereby it has low calorie value. Hanson *et al* (2006) analyzed 35 brinjal genotypes and reported sugar content in the range of 20-30 % on dry weight basis. In present study the total soluble sugars content was found in the range of 0.53-24.77 % in 2012 and in the range of 0.71-20.36 % in 2013 (Table 8). In 2012, the average total soluble sugars content was 10.02 % while in 2013, the average content was found to be 9.89 %. Genotype G-418 showed minimum content (0.53 %) in 2012 while genotype G-412 (0.71%) showed minimum total soluble sugars in 2013. Fig 5 represents the distribution of total soluble sugars in brinjal genotypes. Maximum numbers of genotypes were having total soluble sugars in the range of 5-10 % in 2012 and 2013. In 2012, 16 genotypes showed total soluble sugars in this range while in 2013, 17 genotypes showed the content in this range. Table 9 shows elite genotypes having total soluble sugars in the range of 5-10 %. According to Choudhury (1976), white brinjal is considered good for diabetic

Table 5: Anthocyanins content in whole fruit of various brinjal genotypes in 2012 and 2013.

Name of the Genotype	Colour of the genotype	Anthocyanin content (mg/100g)		Mean
		2012	2013	
BL-2001-1-2	White	4.91±0.6	4.59±0.5	**4.75**
BL-201	Purple	10.45±2.4	10.21±2.6	**10.33**
BL-202	Purple	24.06±0.1	25.09±0.0	**24.57**
BL-204	Purple	14.09±0.1	13.99±0.1	**14.04**
BL-207	Purple	42.35±4.6	41.88±4.5	**42.11**
BL-214	Purple	5.09±0.2	6.48±0.1	**5.78**
BL-215	Purple	46.81±4.9	47.31±5.0	**47.06**
BL-219	Purple	45.37±3.9	46.83±3.9	**46.10**
BL-220	Purple	12.18±0.1	10.79±0.2	**11.58**
BLW-231	White	5.47±1.0	4.97±0.7	**5.22**
BLEND-11-WR-1	White	4.22±0.5	3.69±0.4	**3.95**
BLEND-11-WR-2	White with purple streaks	4.12±0.4	3.43±0.4	**3.78**
SR-308	Green	9.63±0.6	7.73±0.4	**8.68**
BR-104	Purple	26.33±0.6	25.75±0.4	**26.04**
BR-118	Purple	72.41±2.23	71.31±2.0	**71.85**
BR-133	Purple	9.37±0.1	9.83±0.1	**9.60**
BRG-111	Pink	1.52±0.1	2.86±0.0	**2.19**
BRG-224	Pink	15.18±0.1	12.21±0.3	**13.69**
G-401	Green	4.56±1.2	2.60±1.6	**3.58**
G-402	Green	6.07±0.2	5.17±1.1	**5.62**
G-403	Green	8.09±0.1	5.79±0.1	**6.94**
G-405	Green	4.96±0.1	5.15±0.2	**5.06**
G-407	Green	3.39±0.6	3.04±0.5	**3.22**
G-408	Green	4.10±0.7	2.16±0.8	**3.13**
G-409	Green	3.97±0.1	4.09±0.2	**4.03**

G-411	Green	30.98±0.1	30.78±0.1	**30.88**
G-412	Green	0.55±0.3	1.87±0.3	**1.21**
G-414	Green	4.09±0.4	4.08±0.3	**4.09**
G-415	Green	9.22±1.0	7.44±1.0	**8.33**
G-418	Green	5.06±0.1	5.02±0.1	**5.04**
MR-319	Purple	32.37±0.8	29.51±0.5	**30.94**
MR-320	Purple	22.96±0.6	22.62±0.6	**22.79**
P-71	Purple	3.28±0.1	4.25±0.7	**3.77**
SR-301	Purple	19.20±0.9	18.89±0.8	**19.04**
SR-302	Purple	12.60±0.6	12.25±0.8	**12.43**
SR-303	Purple	88.24±4.6	88.91±4.6	**88.57**
SR-304	Purple	15.38±0.2	13.61±0.3	**14.49**
SR-305	Purple	19.53±0.9	19.03±0.9	**19.28**
SR-306	Purple	23.08±0.1	22.03±0.2	**22.55**
SR-307	Purple	20.08±0.1	18.38±0.3	**19.23**
SR-309	Purple	22.56±0.2	19.77±0.7	**21.16**
SR-310	Purple	11.73±0.1	10.97±0.5	**11.35**
SR-311	Purple	25.55±0.4	25.73±0.3	**26.64**
SR-312	Purple	42.40±2.6	39.55±2.4	**40.97**
SR-313	Purple	54.89±0.6	54.02±0.7	**54.46**
SR-318	Purple	66.20±0.3	63.81±0.6	**65.00**
SRV-360-1	White	8.57±0.1	6.30±0.0	**7.43**
W-230-42-45-1	White	6.49±0.0	7.24±0.6	**6.86**
WL-502	White	6.58±1.0	6.05±0.6	**6.31**
WO-406	White	4.03±0.5	3.46±0.4	**3.74**
	MEAN	**18.93**	**18.25**	
CD (5 %)	A = Genotypes = 2.08 B = Anthocyanin content = 0.42 AxB = NS			

NS = Non-Significant

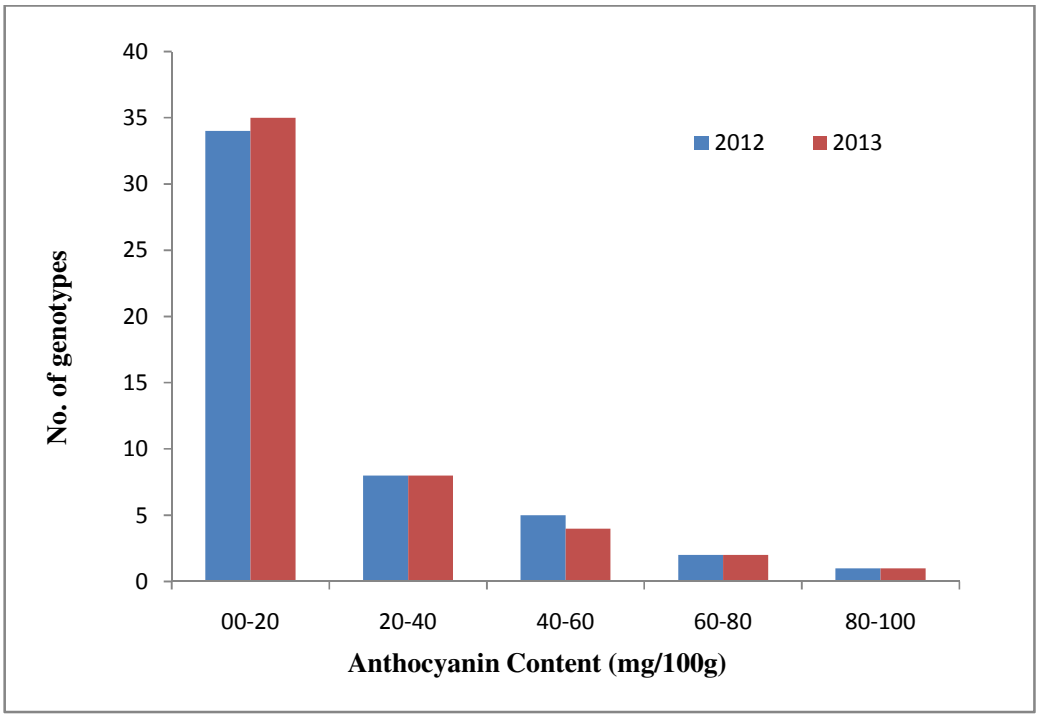


Fig 3: Frequency histogram showing distribution of anthocyanins (mg/100g) in whole fruit of various genotypes of brinjal in 2012 and 2013.

Table 6: Elite genotypes having anthocyanin content > 60 mg/100g in whole fruit of various brinjal genotypes in 2012 and 2013

Genotypes in 2012	Genotypes in 2013
BR-118	BR-118
SR-303	SR-303
SR-318	SR-318

Table 7: Variation in average anthocyanin content of brinjal in different plant parts in 2012 and 2013.

Brinjal plant parts	No. of genotypes	Anthocyanins content (MEAN±S.E.)	
		2012	2013
Peel	50	59.89±6.86	59.12±6.94
Flesh	50	1.02±0.22	1.13±0.17
Whole fruit	50	18.93±2.80	18.25±2.78

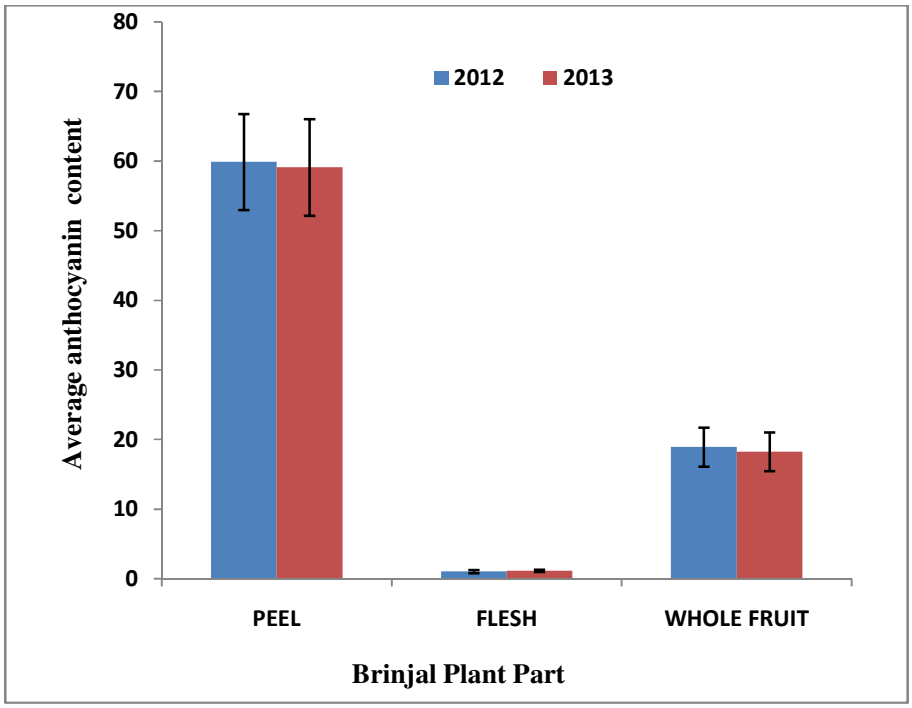


Fig 4: Frequency histogram showing average content of anthocyanins (mg/100g) in peel, flesh and whole fruit of various genotypes of brinjal in 2012 and 2013.

Table 8 : Total soluble sugars content in various brinjal genotypes in 2012 and 2013

Name of the Genotype	Colour of the genotype	Total soluble sugars (%)		Mean
		2012	2013	
BL-2001-1-2	White	7.60±0.14	8.14±0.04	**7.81**
BL-201	Purple	19.77±0.12	15.62±1.02	**17.69**
BL-202	Purple	19.59±0.44	17.33±0.00	**18.46**
BL-204	Purple	9.63±0.82	6.50±0.03	**8.06**
BL-207	Purple	9.13±0.05	12.56±0.01	**10.85**
BL-214	Purple	9.87±0.05	5.76±0.03	**7.81**
BL-215	Purple	18.03±0.05	16.88±1.14	**17.46**
BL-219	Purple	17.23±0.12	14.44±0.00	**15.84**
BL-220	Purple	24.77±0.33	11.74±0.04	**18.25**
BLW-231	White	9.87±0.47	6.79±0.01	**8.33**
BLEND-11-WR-1	White	ND	4.79±0.01	**2.40**
BLEND-11-WR-2	White with purple streaks	6.27±0.29	5.45±0.02	**5.86**
SR-308	Green	9.86±0.48	7.13±0.07	**8.49**
BR-104	Purple	14.97±0.01	8.10±0.01	**11.53**
BR-118	Purple	16.08±0.32	19.51±0.65	**17.79**
BR-133	Purple	7.39±0.03	15.80±0.92	**16.59**
BRG-111	Pink	7.77±0.12	4.77±0.01	**6.27**
BRG-224	Pink	13.13±0.05	10.11±0.81	**11.62**
G-401	Green	12.30±0.00	13.30±0.06	**12.80**
G-402	Green	14.78±0.26	10.37±0.04	**12.58**
G-403	Green	2.87±0.34	4.21±0.57	**3.54**
G-405	Green	13.80±0.08	9.44±0.04	**11.62**
G-407	Green	3.83±0.09	4.62±0.78	**4.22**
G-408	Green	14.80±0.23	12.12±0.08	**13.46**
G-409	Green	24.71±0.22	16.13±0.03	**20.42**

G-411	Green	5.10±0.50	9.56±0.00	**7.33**
G-412	Green	0.70±0.22	0.71±0.01	**0.70**
G-414	Green	10.38±0.32	10.57±0.02	**10.47**
G-415	Green	4.93±0.54	2.35±0.02	**3.64**
G-418	Green	0.53±0.17	0.88±0.01	**0.71**
MR-319	Purple	2.75±0.01	4.92±0.51	**3.83**
MR-320	Purple	3.72±0.01	8.62±0.05	**6.17**
P-71	Purple	5.00±0.14	4.74±1.03	**4.87**
SR-301	Purple	5.26±0.05	7.65±0.02	**6.46**
SR-302	Purple	7.65±0.05	5.47±0.03	**6.56**
SR-303	Purple	5.43±0.34	7.32±0.09	**6.38**
SR-304	Purple	3.00±0.14	4.70±0.01	**3.85**
SR-305	Purple	5.23±0.09	6.50±0.06	**5.87**
SR-306	Purple	14.03±0.39	14.80±0.02	**14.41**
SR-307	Purple	13.00±0.00	10.54±0.02	**11.77**
SR-309	Purple	19.84±0.47	15.21±0.01	**17.52**
SR-310	Purple	15.00±0.51	10.83±0.53	**12.91**
SR-311	Purple	9.40±0.00	7.97±0.04	**8.69**
SR-312	Purple	14.99±0.38	11.46±0.02	**13.22**
SR-313	Purple	22.91±0.23	20.36±0.03	**21.63**
SR-318	Purple	6.43±0.19	8.84±0.05	**7.64**
SRV-360-1	White	1.13±0.05	2.61±0.01	**1.87**
W-230-42-45-1	White	5.74±1.00	7.00±0.00	**6.37**
WL-502	White	ND	3.15±0.05	**1.58**
WO-406	White	1.01±0.13	0.81±0.01	**0.91**
MEAN		**10.02**	**8.98**	
CD (5 %)		A = Genotypes = 0.51 B = Total soluble sugars = 0.10 AxB = 0.73		

ND = Not Detected

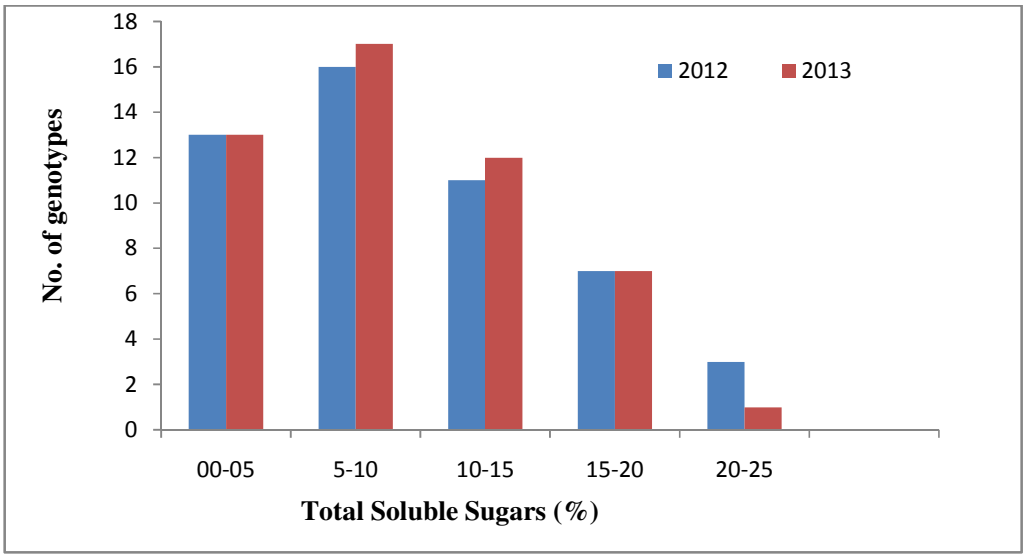


Fig 5: Frequency histogram showing distribution of sugars (%) in various genotypes of brinjal in 2012 and 2013.

Table 9: Elite genotypes showing total soluble sugars in the range 5-10 % in various genotypes of brinjal in 2012 and 2013.

Genotypes in 2012	Genotypes in 2013
BL-204	BL-204
BL-207	BL-214
BL-214	BLW-231
BLW-231	BLEND-11-WR-2
BLEND-11-WR-2	SR-308
SR-308	G-405
BR-133	G-411
BRG-111	MR-320
G-411	SR-301
SR-301	SR-302
SR-302	SR-303
SR-303	SR-305
SR-305	W-230-42-45-1
SR-311	SR-311
SR-318	SR-318
	BR-104

patients. The present study confirms this, as in this study genotypes having white fruits such as BLEND-11-WR-1, BLEND-11-WR-2, SRV-360-1, W-502 and WO-406 showed low total soluble sugars content and could be fit for diabetic patients. Thaipong *et al* (2006) also reported total phenol content in the range from 170.0 to 300.8 mg/100g in guava with pink pulp. Kaur *et al* (2014) showed a wide variation in total phenols in brinjal genotypes, ranging

from 22.62 to 234.46 mg /100g FW (244.28 to 2990.64 mg /100g DW) having nearly 15 fold variations. In present study the total phenols content varied in the range of 70.21 to 296.37 mg/100g DW with average value of 125.58 mg/100g in 2012 and in the range of 75.16 to 298.63 mg/100g DW with average value of 120.78 mg/100g in 2013 (Table 10). High diversity within the species for phenolics concentration also demonstrated by Stomell and Whitaker (2003), Hanson *et al* (2006) and Prohens *et al* (2005). These values are in agreement with Hanson *et al* (2006) who proposed that the environmental differences among years have influence on the concentrations of phenolics. In present study maximum amount of total phenols was found in G-415 (about 300 mg/100g) in two years. Patel *et al* (2013) also reported a higher total phenolic content in green variety (KH1, 490.96 mg/100g) than purple variety (Dolly 5, 325.69 mg/100g). This showed that genotypes having green fruits have high content of total phenols which have high free radical scavenging activity (Lutharia and Mukhopadhay 2006). The distribution of total phenols in investigated brinjal genotypes is shown in Fig 6. The maximum number of genotypes showed total phenols in the range of 100-150 mg/100g in both 2012 and 2013. Only one genotype showed total phenols in the maximum range of 250-300 mg/100g in both years. Table 11 shows the elite genotypes having > 200 mg/100g total phenols content. Three genotypes G-412, G-414 and G-415 showed the total phenols >200 mg/100g which are again in agreement with results obtained by Patel *et al* (2013).

The ODHs varied from 3.41 to 93.00 mg/100g having highest content in genotype P-71 (93.00±4.6 mg/100g) in 2012 as in 2013, the ODHs varied in the range of 3.69-98.72 (mg/100g) having highest content in P-71 (98.72±0.1 mg/100g) (Table 12). The average content of ODH found was 37.73 mg/100g DW in 2012 while in 2013 it was 35.80 mg/100g. Fig 7 shows the distribution of ODHs in various brinjal genotypes. Maximum number of genotypes showed ODH content in the range of 20-40 mg/100g. G-418 and P-71 showed ODH content in the maximum range (80-100 mg/100g). Elite genotypes which showed ODH content >60 mg/100g are shown in Table 13. Table 13 shows that genotypes having green fruits have high content of ODHs.

Flavonols in brinjal were found in the range 25.17 to 847.78 mg/100g in 2012 and between 21.38-835.86 mg/100g in 2013 which showed about 34 fold variation (Table 14). The average flavonol content in 2012 was 234.29 mg/100g with highest content in BLEND-11-WR-2 (848.99±7.22 mg/100g) and in 2013 it was 228.19 mg/100g with highest amount of flavonols found in genotype BLEND-WR-11-2 (835.86±16.9 mg/100g). The distribution of flavonols is shown in Fig 8. The maximum number of genotypes showed flavonol content in the range of 0-200 mg/100g while the maximum range was 800-1000 mg/100g. Helmja *et al* (2007) reported that brinjal has the highest amount of flavonols (660 mg/100g) among Solanaceae family. Table 15 shows the genotypes having flavonol content > 600 mg/100g in

Table 10: Total phenols content in various brinjal genotypes in 2012 and 2013

Name of the Genotype	Colour of the genotype	Total Phenols (mg/100g)		MEAN
		2012	2013	
BL-2001-1-2	White	125.95±0.5	111.15±0.3	**118.55**
BL-201	Purple	129.57±1.0	119.89±0.1	**124.73**
BL-202	Purple	115.42±0.1	116.95±0.1	**116.18**
BL-204	Purple	113.42±2.7	109.84±0.1	**111.62**
BL-207	Purple	149.76±0.9	122.52±0.2	**136.14**
BL-214	Purple	102.44±0.9	106.734±0.2	**104.59**
BL-215	Purple	72.21±2.6	96.15±1.2	**84.18**
BL-219	Purple	100.37±0.26	125.63±0.1	**112.10**
BL-220	Purple	129.15±0.3	143.84±0.1	**136.50**
BLW-231	White	111.79±4.0	123.15±0.0	**117.47**
BLEND-11-WR-1	White	98.65±2.7	87.42±0.2	**93.03**
BLEND-11-WR-2	White with purple streaks	118.57±0.4	140.41±0.1	**129.49**
SR-308	Green	106.22±1.2	91.31±0.2	**98.77**
BR-104	Purple	70.21±0.5	94.04±0.5	**82.12**
BR-118	Purple	124.31±2.0	106.07±2.0	**115.19**
BR-133	Purple	137.84±4.2	122.13±0.1	**130.05**
BRG-111	Pink	155.47±3.4	140.63±0.1	**148.06**
BRG-224	Pink	83.73±0.9	108.05±0.1	**95.89**
G-401	Green	134.79±1.8	112.63±0.2	**123.71**
G-402	Green	132.42±2.4	100.10±0.2	**116.26**
G-403	Green	149.36±1.3	123.47±0.4	**136.41**
G-405	Green	129.89±1.3	111.47±0.2	**120.68**
G-407	Green	92.05±1.5	75.16±0.2	**83.60**
G-408	Green	129.89±3.1	101.68±0.2	**115.79**
G-409	Green	98.82±0.9	75.79±0.3	**87.30**

G-411	Green	136.36±0.3	110.42±0.1	**123.39**
G-412	Green	222.31±1.8	219.47±0.9	**220.89**
G-414	Green	207.10±1.1	220.70±9.7	**213.90**
G-415	Green	293.52±0.4	298.63±9.5	**296.07**
G-418	Green	114.94±1.4	125.37±0.2	**120.15**
MR-319	Purple	121.62±0.3	110.31±0.9	**115.97**
MR-320	Purple	126.79±0.5	125.37±0.2	**126.08**
P-71	Purple	118.47±2.2	135.15±0.2	**126.81**
SR-301	Purple	139.73±0.4	122.26±0.1	**130.10**
SR-302	Purple	87.70±5.2	92.37±0.0	**90.03**
SR-303	Purple	107.84±2.6	97.89±0.0	**102.87**
SR-304	Purple	135.63±2.5	116.26±0.2	**125.95**
SR-305	Purple	119.61±1.6	115.42±0.2	**117.51**
SR-306	Purple	148.55±2.2	120.63±0.2	**134.59**
SR-307	Purple	90.47±5.1	120.94±0.2	**105.71**
SR-309	Purple	78.45±1.2	96.21±1.2	**87.33**
SR-310	Purple	124.14±1.6	110.84±0.2	**117.48**
SR-311	Purple	119.21±0.9	120.94±0.2	**120.07**
SR-312	Purple	125.05±1.0	115.26±0.0	**120.15**
SR-313	Purple	146.57±1.5	134.38±0.2	**140.71**
SR-318	Purple	121.15±0.8	139.84±0.1	**130.49**
SRV-360-1	White	133.58±1.1	110.47±0.1	**122.02**
W-230-42-45-1	White	102.79±0.1	75.52±0.2	**89.15**
WL-502	White	120.68±1.1	123.26±0.1	**121.97**
WO-406	White	124.52±0.6	114.47±0.2	**119.50**
MEAN		**125.58**	**120.78**	
CD (5 %)		A = Genotypes = 2.55 B = total phenols = 0.51 AxB = 3.60		

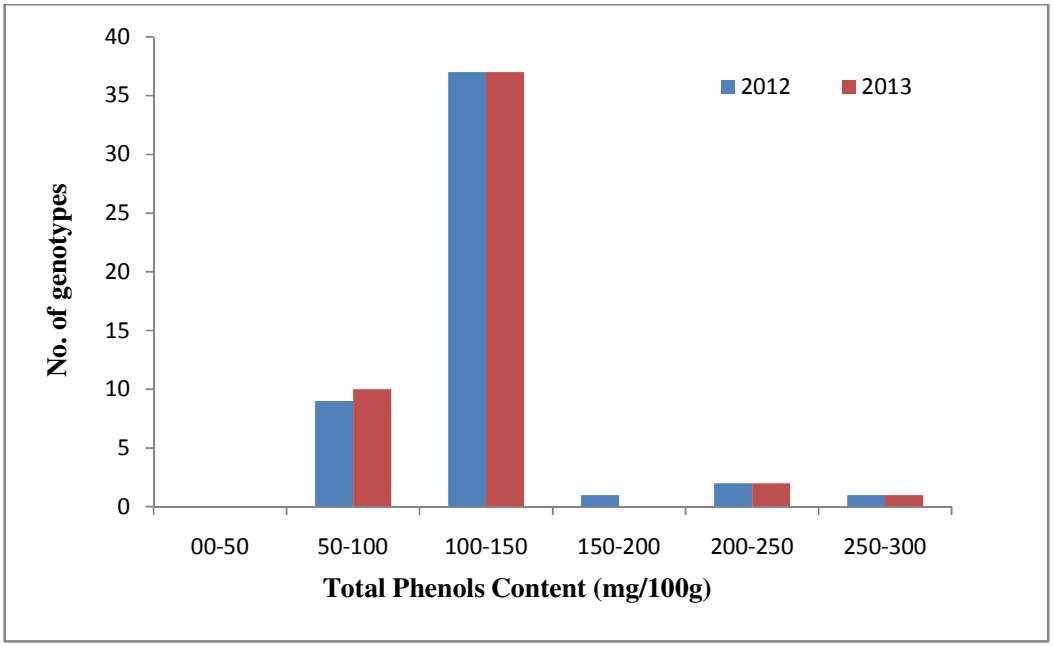


Fig 6: Frequency histogram showing distribution of total phenols (mg/100g) in various genotypes of brinjal in 2012 and 2013.

Table 11: Elite genotypes having total phenol content >200 mg/100g DW of various brinjal genotypes in 2012 and 2013.

Genotypes in 2012	Genotypes in 2013
G-412	G-412
G-414	G-414
G-415	G-415

Table 12: Ortho-dihydroxy phenols content in various genotypes of brinjal in 2012 and 2013.

Name of the Genotype	Colour of the genotype	ODH Content (mg/100g)		MEAN
		2012	2013	
BL-2001-1-2	White	10.93±1.5	13.96±2.3	**12.45**
BL-201	Purple	35.59±2.1	25.76±0.1	**30.67**
BL-202	Purple	39.31±0.3	41.41±0.1	**40.36**
BL-204	Purple	30.10±3.3	44.41±0.1	**37.26**
BL-207	Purple	15.31±4.3	30.38±0.1	**22.85**
BL-214	Purple	59.03±1.9	42.35±0.1	**55.69**
BL-215	Purple	39.62±2.4	39.74±0.2	**39.68**
BL-219	Purple	29.79±0.3	19.00±0.2	**24.40**
BL-220	Purple	39.66±0.8	32.66±0.3	**36.16**
BLW-231	White	39.61±0.7	38.59±0.4	**39.10**
BLEND-11-WR-1	White	24.47±2.6	23.35±0.1	**23.91**
BLEND-11-WR-2	White with purple streaks	31.59±2.0	32.35±0.3	**31.97**
SR-308	Green	39.90±3.3	30.86±0.2	**35.38**
BR-104	Purple	4.03±3.4	3.69±0.1	**3.86**
BR-118	Purple	35.59±2.1	39.83±1.4	**37.71**
BR-133	Purple	6.55±1.7	15.52±0.0	**11.03**
BRG-111	Pink	13.68±3.4	15.45±0.1	**14.56**
BRG-224	Pink	57.55±0.3	59.92±1.4	**59.74**
G-401	Green	41.28±3.3	41.45±0.7	**41.36**
G-402	Green	21.62±3.3	24.27±0.2	**22.95**
G-403	Green	45.86±2.1	21.31±0.3	**33.59**
G-405	Green	31.31±0.7	29.41±0.1	**30.36**
G-407	Green	62.52±2.9	60.11±0.9	**61.31**
G-408	Green	59.28±0.1	41.10±0.3	**56.19**
G-409	Green	58.83±0.2	42.90±0.1	**50.91**

G-411	Green	58.83±0.3	40.69±0.7	**49.76**
G-412	Green	30.07±0.5	24.80±0.1	**27.43**
G-414	Green	26.87±1.1	23.28±0.0	**25.07**
G-415	Green	70.04±0.7	67.52±0.3	**68.78**
G-418	Green	82.11±0.1	84.38±0.3	**83.24**
MR-319	Purple	33.96±1.5	38.93±0.2	**36.45**
MR-320	Purple	28.14±0.7	28.66±0.2	**28.40**
P-71	Purple	93.00±4.6	98.72±0.1	**95.86**
SR-301	Purple	24.10±0.1	28.55±0.1	**26.33**
SR-302	Purple	20.62±4.6	33.24±0.2	**26.93**
SR-303	Purple	40.31±2.4	39.97±0.2	**40.14**
SR-304	Purple	53.00±4.2	48.62±0.2	**50.81**
SR-305	Purple	49.52±2.7	40.55±0.3	**45.04**
SR-306	Purple	58.81±0.5	40.35±0.2	**49.58**
SR-307	Purple	25.35±3.9	38.95±0.5	**32.15**
SR-309	Purple	37.97±0.6	39.81±0.8	**38.89**
SR-310	Purple	35.93±0.7	24.76±0.1	**30.35**
SR-311	Purple	59.48±0.4	48.52±0.1	**54.00**
SR-312	Purple	37.69±0.1	38.76±0.1	**38.22**
SR-313	Purple	39.85±2.9	23.28±0.4	**31.56**
SR-318	Purple	3.41±0.5	9.04±0.1	**6.23**
SRV-360-1	White	23.40±1.8	23.52±0.3	**23.46**
W-230-42-45-1	White	37.04±4.8	51.14±0.2	**44.09**
WL-502	White	11.33±2.5	13.66±0.1	**12.49**
WO-406	White	32.50±2.2	30.41±0.1	**31.46**
MEAN		**37.73**	**35.80**	
CD (5 %)		A = Genotypes = 1.95 B = Ortho-dihydroxy phenol content = 0.39 AxB = 2.75		

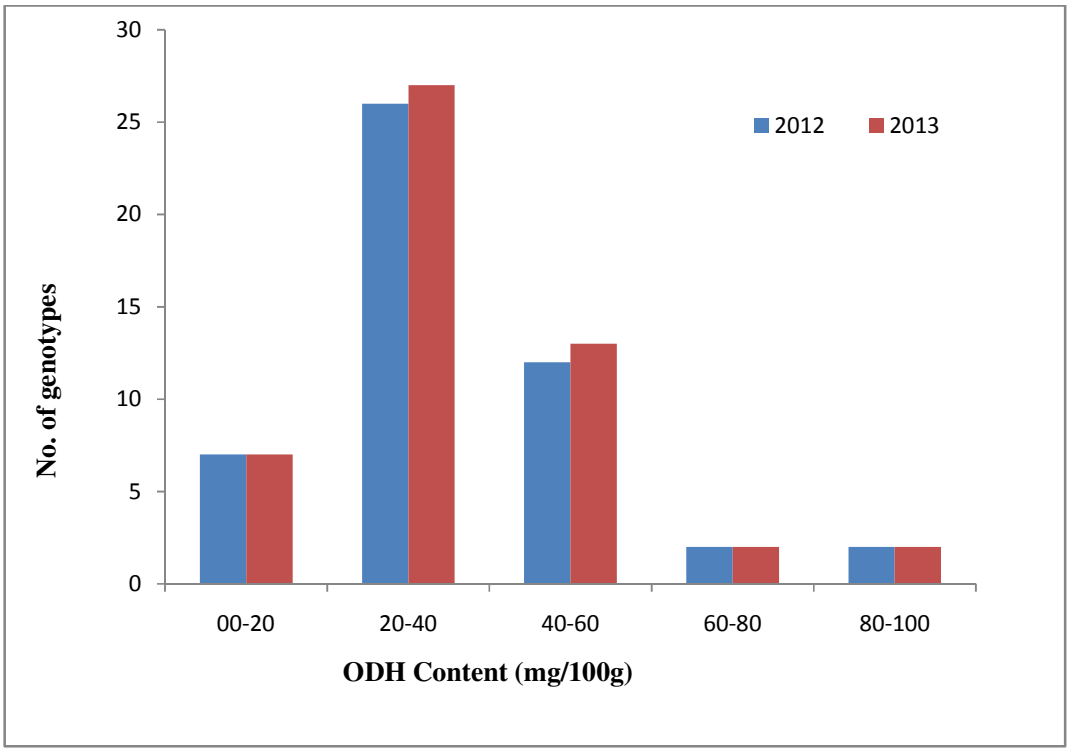


Fig 7: Frequency histogram showing distribution of ODHs (mg/100g) in various genotypes of brinjal in 2012 and 2013.

Table 13: Elite genotypes of brinjal showing ODH content > 60 mg/100g in 2012 and 2013.

Genotypes in 2012	Genotypes in 2013
G-407	G-407
G-415	G-415
G-418	G-418
P-71	P-71

Table 14: Flavonol content of various genotypes of brinjal in 2012 and 2013.

Name of the Genotype	Colour of the genotype	Flavonols (mg/100g)		MEAN
		2012	2013	
BL-2001-1-2	White	257.79±3.5	249.48±0.5	**253.54**
BL-201	Purple	363.43±2.1	353.27±1.3	**358.35**
BL-202	Purple	185.34±1.0	197.41±0.5	**196.38**
BL-204	Purple	133.16±2.7	130.86±1.4	**132.01**
BL-207	Purple	134.02±6.2	129.48±0.3	**135.75**
BL-214	Purple	351.03±24.6	334.65±0.3	**342.84**
BL-215	Purple	77.07±1.0	46.21±0.3	**61.64**
BL-219	Purple	276.20±0.5	250.86±0.8	**263.53**
BL-220	Purple	206.14±4.5	209.14±0.6	**207.64**
BLW-231	White	133.27±2.7	125.69±1.4	**129.48**
BLEND-11-WR-1	White	359.82±10.5	355.69±0.5	**357.75**
BLEND-11-WR-2	White with purple streaks	848.99±7.22	835.86±16.9	**819.65**
SR-308	Green	99.94±4.2	90.86±0.3	**95.40**
BR-104	Purple	118.10±0.3	126.21±0.5	**122.15**
BR-118	Purple	373.96±10.5	365.00±0.5	**369.48**
BR-133	Purple	346.37±19.6	324.65±0.6	**335.51**
BRG-111	Pink	176.66±2.4	166.72±1.3	**171.69**
BRG-224	Pink	171.20±7.5	163.20±10.6	**167.20**
G-401	Green	71.37±1.8	75.00±0.0	**73.19**
G-402	Green	54.31±1.6	61.55±0.5	**57.93**
G-403	Green	63.73±1.1	62.59±0.5	**63.15**
G-405	Green	155.46±1.7	144.83±0.0	**150.14**
G-407	Green	82.93±6.3	69.83±0.0	**76.38**
G-408	Green	25.17±3.1	21.38±0.8	**23.37**
G-409	Green	32.93±5.2	46.55±0.0	**39.74**

G-411	Green	83.61±9.2	94.08±0.3	**88.85**
G-412	Green	155.46±1.3	143.10±1.6	**149.25**
G-414	Green	84.31±25.4	79.65±1.3	**81.98**
G-415	Green	177.07±4.8	182.41±0.3	**179.73**
G-418	Green	150.45±4.7	139.65±1.0	**145.05**
MR-319	Purple	539.65±0.8	514.14±0.3	**526.89**
MR-320	Purple	742.23±3.9	755.86±0.3	**749.05**
P-71	Purple	189.31±12.0	202.24±0.9	**195.77**
SR-301	Purple	256.55±1.0	216.90±0.3	**236.72**
SR-302	Purple	149.31±3.9	128.97±0.3	**139.14**
SR-303	Purple	155.34±1.9	141.38±0.8	**148.36**
SR-304	Purple	402.86±3.9	395.35±0.3	**399.10**
SR-305	Purple	499.47±5.2	523.45±0.6	**511.46**
SR-306	Purple	582.06±3.2	563.45±1.3	**572.75**
SR-307	Purple	198.97±1.4	195.00±1.4	**196.49**
SR-309	Purple	140.51±0.3	113.79±0.0	**127.15**
SR-310	Purple	195.69±0.9	189.48±0.6	**192.58**
SR-311	Purple	189.48±8.0	167.07±1.4	**178.27**
SR-312	Purple	219.65±4.8	207.41±0.5	**213.53**
SR-313	Purple	394.76±3.1	419.65±0.8	**407.21**
SR-318	Purple	554.13±0.8	525.52±0.5	**539.82**
SRV-360-1	White	182.23±4.7	158.27±2.7	**170.25**
W-230-42-45-1	White	97.46±1.7	107.07±0.6	**102.26**
WL-502	White	162.35±3.0	180.34±0.8	**171.34**
WO-406	White	114.23±0.5	128.10±1.3	**121.17**
MEAN		**234.29**	**228.19**	
CD (5 %)		A = Genotypes = 6.72 B = flavonol content = 1.34 AxB = 9.51		

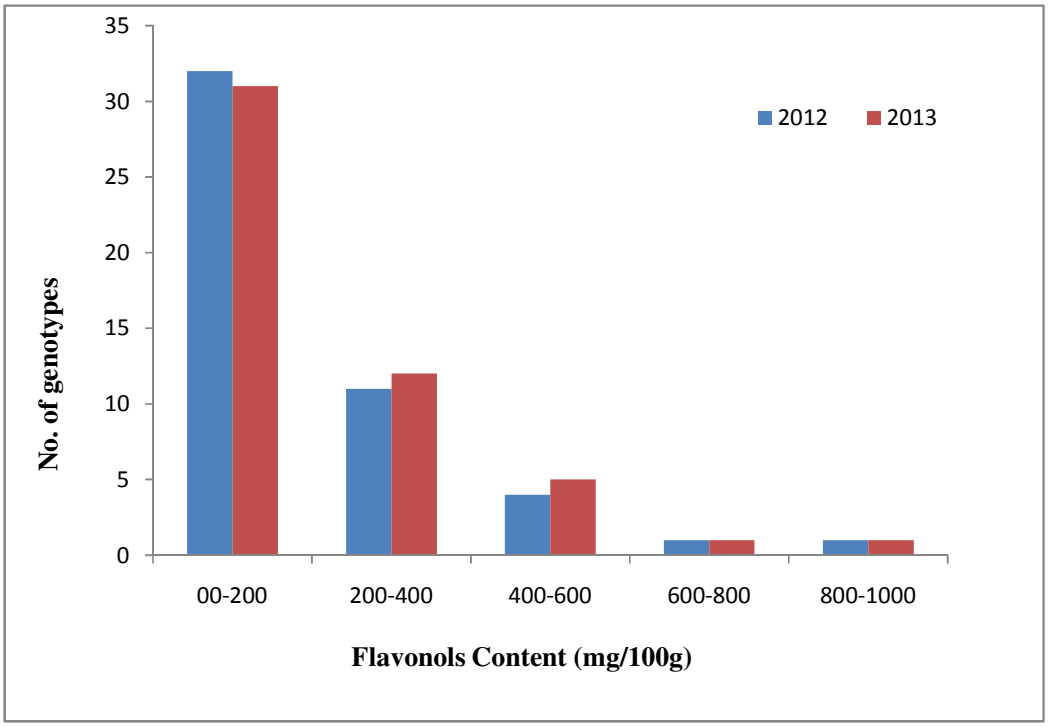


Fig 8: Frequency histogram showing distribution of Flavonols (mg/100g) in various genotypes of brinjal in 2012 and 2013.

Table 15: Elite genotypes showing flavonol content > 600 mg/100g in 2012 and 2013.

Genotypes in 2012	Genotypes in 2013
BLEND-11-WR-2	BLEND-11-WR-2
MR-320	MR-320

brinjal genotypes in 2012 and 2013. Only two genotypes showed content > 600 mg/100g viz. BLEND-11-WR-2 and MR-320.

Correlation among various biochemical parameters in brinjal genotypes

The correlation among various biochemical parameters in 50 genotypes in 2012 and 2013 is shown in Table 16.

In 2012, there was a positive significant (0.607) correlation between anthocyanins present in peel and whole fruit at 1% level of significance. The anthocyanins present in peel, flesh and whole fruit were not dependent on weight of fruit. There was also a significant and positive correlation (0.354) between total soluble sugar content and anthocyanins in peel at 5% level of significance. There was negative correlation among total phenolic content and

anthocyanin in peel. ODH content found was negatively correlated (-0.326) with weight of brinjal fruits at 5% of significant level. Flavonols and anthocyanins in peel were positively correlated (0.333) and significant at 5% level of significance but have no correlation with fruit weight.

In 2013, the anthocyanins present in peel and flesh and total phenols were negatively correlated (-0.330, -0.321 and -0.590) with weight of fruit. There was a positive correlation between anthocyanins present in peel and whole fruit which is significant also. Sugars and anthocyanins in peel and whole fruit were positively correlated to each other. Similar results are showed by Hara *et al* (2003) as they found positive correlation in anthocyanin production and sugar accumulation in radish hypocotyls. No correlation was found between ODHs and anthocyanins present in peel. Similar to the situation in 2012, the flavonols and anthocyanins present in peel and whole fruit were positively correlated.

It was reported that peel tissue of brinjal had higher amount of anthocyanins, phenolics and flavonols than pulp tissue (Tomas-Barberan *et al* 2001; Jhang *et al* 2010). In the present study anthocyanins in peel positively correlated with flavonols while being negatively correlated with total phenols.

Table 16: Correlation among various biochemical parameters in 2012 and 2013.

		Anthocyanins					
	Weight	**Peel**	**Flesh**	**Whole fruit**	**Sugars**	**Total phenols**	**ODHs**
			2012				
Peel	0.086						
Flesh	-0.180	-0.028					
Whole fruit	0.074	0.607**	0.091				
Sugars	0.163	0.354*	-0.086	0.206			
Total phenols	0.105	-0.291*	0.006	-0.161	-0.218		
ODHs	-0.326*	-0.064	0.016	-0.146	-0.036	0.083	
Flavonols	-0.043	0.333*	0.089	0.217	-0.116	0.017	-0.125
			2013				
Peel	-0.330*						
Flesh	-0.321*	0.085					
Whole fruit	-0.177	0.584**	0.098				
Sugars	0.046	0.413	-0.062	0.439**			
Total phenols	-0.590**	-0.149	-0.053	-0.109	-0.221		
ODHs	0.042	0.000	-0.085	-0.126	-0.174	0.060	
Flavonols	-0.163	0.316*	0.234	0.198	0.041	0.900**	-0.133

Where * = significant at $p < 0.05$, ** = significant at $p < 0.01$

Table 17 shows the genotypes having best combination of all studied parameters such as anthocyanin content in peel, flesh and whole brinjal fruit; total soluble sugars, total phenols, ODH and flavonols.

Table 17: Genotypes having best combination of characteristics

	Anthocyanin Content (mg/100g)						
Genotypes	**Peel**	**Flesh**	**Whole fruit**	**Total Soluble Sugars (%)**	**Total Phenols (mg/100g)**	**ODHs (mg/100g)**	**Flavonols (mg/100g)**
BR-118	102.25	0.70	71.85	17.79	115.19	37.71	369.48
MR-319	105.08	1.57	30.94	3.83	115.97	36.45	526.89
MR-320	92.37	1.85	22.79	6.17	126.08	28.40	749.05
SR-303	103.67	1.27	88.57	6.38	102.87	40.14	148.36
SR-304	103.03	1.98	14.49	3.85	125.95	50.81	399.10
SR-306	99.76	3.78	22.55	11.77	105.71	32.15	196.49
SR-310	105.76	1.84	26.64	12.91	117.48	30.35	192.58
SR-311	104.88	1.18	26.64	8.96	120.07	54.00	178.27
SR-312	110.75	1.90	40.97	13.22	120.15	38.22	213.53
G-411	45.79	0.98	30.88	7.33	123.90	49.76	88.85

4.2 ENZYMATIC STUDIES ON SELECTED GENOTYPES FOR ANTHOCYANINS SYNTHESIZING ENZYMES IN LEAVES AND FRUITS AT VARIOUS STAGES OF FRUIT RIPENING

The anthocyanin synthesizing pathway is one of the most extensively studied pathways of plant secondary metabolites (Heller and Forkmann 1993). In higher plants anthocyanins are synthesized via phenylpropanoid pathway (Williams and Grayer 2004). The first step of this pathway is catalyzed by enzyme phenylalanine ammonia lyase (PAL) which converts phenylalanine to *trans*-cinnamic acid by the elimination of ammonia. The *trans*-cinnamic acid is further hydroxylated to form p-coumaric acid. Tyrosine ammonia lyase (TAL) involved in direct formation of p-coumaric acid from tyrosine. p-coumaric acid is the precursor of 4-coumaroyl-CoA which serves as a substrate for the synthesis of anthocyanins along with other flavonoid derivatives (Ehlting *et al* 1999). In the pathway of anthocyanins synthesis anthocyanidin synthase (ANS) is postulated to catalyze the conversion of colourless leucoanthocyanidins to coloured anthocyanidins. Leucoanthocyanidin is the natural substrate of ANS but it is unstable in nature. So the ANS activity was analyzed by conversion of dihydroquercetin (DHQ) to quercetin which is a flavonol (Turnbull *et al* 2004).

In present research after the analysis of anthocyanins content, total soluble sugars and phenolic compounds in 50 genotypes, fourteen genotypes were selected for the assay of enzyme ANS along with PAL and TAL. It is known that the purple genotypes have higher anthocyanins than green and white, but in this study genotypes selected for investigation also included the green and white colour genotypes to compare the synthesis of anthocyanins in brinjal genotypes having different colour. The selected genotypes included BR-118 (Purple), BL-204 (Purple), BL-207 (Purple), BL-214 (Purple), BL-215 (Purple), BL-220 (Purple), BLW-231 (White), BR-104 (Purple), BR-133 (Purple), G-418 (Green), P-71 (Purple), SR-305 (Purple), SR-306 (Purple) and SRV-360-1 (White). The activity of three enzymes was studied at 7, 14 and 21 days after anthesis (DAA).

4.2.1 Activity of ANS Enzyme

Leaves: Variation in ANS activity in leaves of brinjal plant at different stages after anthesis is given in Table 18. Leaf extracts of brinjal genotypes showed decrease in ANS activity from 7 days to 14 days followed by a decrease in activity till 21 days. The maximum activity attained was 28.83 $\delta A\ min^{-1}\ g^{-1}$ fresh tissues in BL-204 at 7 days (Fig 9). The genotype P-71 also showed high activity (25.33 $\delta A\ min^{-1}g^{-1}$ fresh tissue) at 7 days.

Fruits: Variation in ANS activity in brinjal fruits is represented in Table 19. Activity of ANS in fruits was less as compared to that in leaves. The maximum activity was shown by genotype SR-305 (12.00 $\delta A\ min^{-1}g^{-1}$ fresh tissue) at 21 days (Fig 10).

Saito *et al* (1999) reported the accumulation of tissue specific form of ANS protein correlated with anthocyanin accumulation. The present study is in agreement with above as there was decrease in activity in leaves having low amount of anthocyanins while increase in ANS activity in fruits having high anthocyanin content was observed. The ANS activity increased during growth of fruits with purple colour and it was maximum at 21 days characterized by deep purple colour while a decrease in ANS activity was observed in green and white genotypes. The genotypes G-418 (green), BLW-231 and SRV-360-1 (white) showed decrease in ANS activity from 7 days to 21 days. Koes *et al* (1994) also reported the correlation between accumulation of anthocyanins and activity of the enzymes involved in their synthesis. The activity of ANS involved in the biosynthetic pathway of anthocyanins follows the same pattern as that of the accumulation of anthocyanins and flavonoids (Reddy *et al* 2007). Our data presented similar results to the above, that is decrease in ANS activity in leaves and green and white fruits of brinjal with development and increase in ANS activity in purple fruits with the increase in anthocyanins.

4.2.2 Activity of PAL enzyme

Leaves: The enzymatic extracts of leaves of *Solanum melongena* L. genotypes showed a steep increase in PAL activity till 14 days and then decrease till 21 days. The maximum activity attained was 61.00 µmoles cinnamic acid formed $min^{-1}\ g^{-1}$ fresh tissue at 14

Table 18: Anthocyanidin Synthase (ANS) activity in leaves of brinjal at various stages of growth

Name of the Genotype	Colour of the Genotype	Day After Anthesis (DAA)			MEAN
		7	14	21	
BR-118	Purple	6.33±1.04 (0.520±0.25)	1.00±0.25 (0.002±0.20)	0.77±0.25 (0.001±0.30)	**2.70**
BL-204	Purple	28.83±1.40 (4.133±0.01)	20.67±0.76 (2.043±0.06)	6.17±0.29 (0.510±0.09)	**18.56**
BL-207	Purple	2.67±0.29 (0.010±0.09)	1.50±0.30 (0.003±0.03)	0.50±0.25 (0.001±0.01)	**1.56**
BL-214	Purple	4.50±0.50 (0.027±0.05)	2.50±0.50 (0.010±0.01)	0.47±0.50 (0.001±0.05)	**2.49**
BL-215	Purple	2.50±0.30 (0.010±0.05)	1.00±0.33 (0.002±0.02)	0.83±0.29 (0.001±0.01)	**1.45**
BL-220	Purple	10.17±1.93 (1.027±0.01)	0.50±0.20 (0.001±0.01)	0.50±0.10) (0.001±0.01)	**3.72**
BLW-231	White	7.17±1.61 (0.980±0.40)	5.50±0.50 (0.250±0.05)	2.00±0.50 (0.003±0.05)	**4.89**
BR-104	Purple	1.50±0.50 (0.003±0.05)	1.00±0.40 (0.002±0.02)	0.50±0.45 (0.001±0.01)	**1.00**
BR-133	Purple	1.50±0.50 (0.003±0.03)	1.00±0.40 (0.002±0.02)	0.50±0.45 (0.001±0.01)	**1.00**
G-418	Green	13.50±1.32 (2.04±0.02)	3.00±0.30 (0.010±0.01)	2.50±0.25 (0.010±0.01)	**6.33**
P-71	Purple	25.33±1.51 (3.050±0.09)	4.67±0.29 (0.020±0.01)	3.00±0.25 (0.010±0.01)	**11.00**
SR-305	Purple	1.50±0.30 (0.003±0.03)	1.17±0.29 (0.002±0.01)	0.50±0.28 (0.001±0.01)	**1.05**
SR-306	Purple	4.67±0.58 (0.023±0.01)	1.5±0.50 (0.003±0.03)	1.00±0.45 (0.002±0.02)	**2.39**
SRV-360-1	White	13.50± 1.32 (2.093±0.01)	2.50±0.50 (0.010±0.05)	1.00±0.50 (0.002±0.02)	**5.67**
MEAN		**8.33**	**3.93**	**1.45**	
CD (5%)		**A** = Genotypes = 1.177 **B** = DAA = 0.545 **AxB** = 2.038			

Anthocyanidin Synthase (ANS) activity is expressed as change in absorbance min^{-1} g^{-1} FW

Values in the parenthesis indicate specific activity (change in absorbance/ min/ mg protein) of the enzyme

Values are mean±S.D. of the three replications

Table 19: Anthocyanidin Synthase (ANS) enzymatic activity in fruit at various stages of growth of brinjal.

Name of the Genotype	Colour of the Genotype	Day After Anthesis (DAA)			MEAN
		7	14	21	
BR-118	Purple	0.83±0.29 (0.003±0.01)	1.00±0.29 (0.010±0.01)	1.83±0.50 (0.016±0.01)	**1.22**
BL-204	Purple	1.17±0.20 (0.013±0.02)	1.67±0.30 (0.015±0.03)	4.00±0.50 (0.070±0.05)	**2.28**
BL-207	Purple	2.83±0.76 (0.056±0.06)	4.00±0.45 (0.070±0.05)	4.50±0.29 (0.720±0.05)	**3.78**
BL-214	Purple	0.50±0.29 (0.003±0.01)	0.77±0.25 (0.004±0.01)	1.83±0.29 (0.010±0.01)	**1.53**
BL-215	Purple	0.50±0.29 (0.003±0.01)	0.83±0.30 (0.004±0.01)	1.67±0.29 (0.010±0.01)	**1.33**
BL-220	Purple	1.00±0.30 (0.010±0.03)	1.17±0.50 (0.013±0.05)	2.17±0.40 (0.050±0.04)	**1.44**
BLW-231	White	3.17±0.29 (0.060±0.01)	2.33±0.29 (0.050±0.01)	1.16±0.29 (0.010±0.01)	**2.22**
BR-104	Purple	0.50±0.00 (0.003±0.01)	0.67±0.29 (0.003±0.01)	1.00±0.29 (0.010±0.01)	**0.72**
BR-133	Purple	2.17± 0.29 (0.050±0.01)	2.67±0.29 (0.053±0.01)	3.33±0.30 (0.060±0.03)	**2.72**
G-418	Green	3.33±0.29 (0.060±0.01)	2.67±0.29 (0.050±0.01)	2.00±0.29 (0.050±0.01)	**2.67**
P-71	Purple	0.83±0.30 (0.004±0.01)	1.00±0.29 (0.005±0.01)	2.10±0.25 (0.056±0.05)	**1.31**
SR-305	Purple	7.00±1.00 (0.100±0.01)	9.50±1.00 (0.227±0.03)	12.00±1.00 (0.323±0.01)	**9.50**
SR-306	Purple	0.53±0.26 (0.003±0.06)	1.67±0.50 (0.010±0.03)	2.17±0.76 (0.050±0.06)	**3.06**
SRV-360-1	White	1.83±0.30 (0.018±0.03)	1.33±0.29 (0.011±0.12)	1.00±0.29 (0.010±0.01)	**1.39**
MEAN		**2.51**	**2.11**	**2.91**	
CD (5%)		**A** = Genotypes = 1.62 **B** = DAA= NS **AxB** = NS			

NS = Non-Significant

Anthocyanidin Synthase (ANS) activity is expressed as change in absorbance min^{-1} g^{-1} FW

Values in the parenthesis indicate specific activity (change in absorbance/ min/ mg protein) of the enzyme

Values are mean±S.D. of the three replications

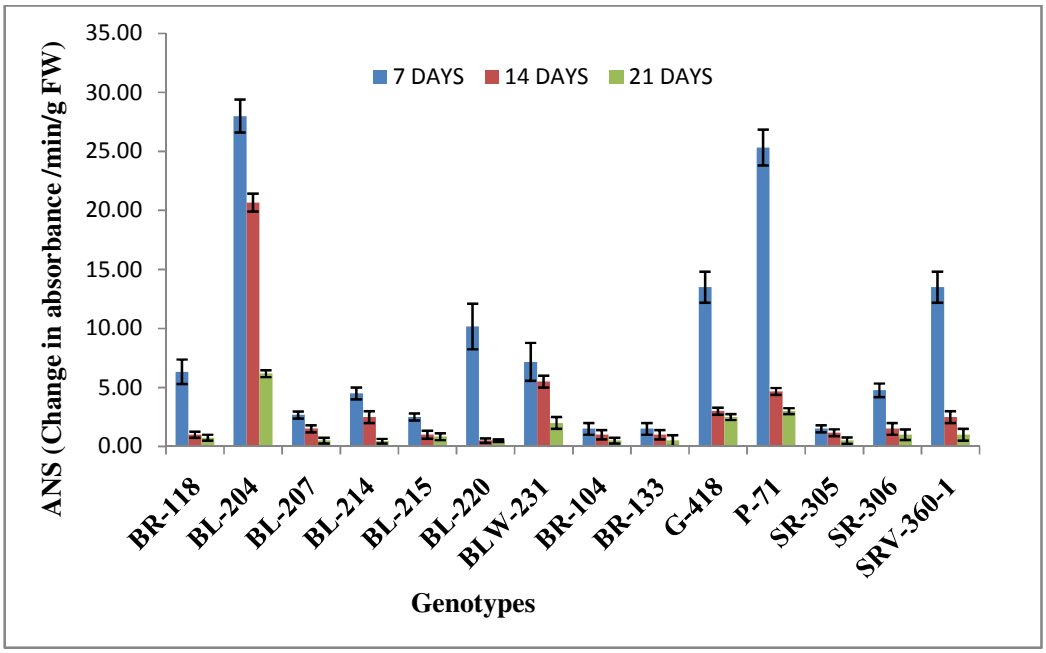


Fig 9: Anthocyanidin Synthase (ANS) activity in leaves of *Solanum melongena* L. genotypes at various days after anthesis.

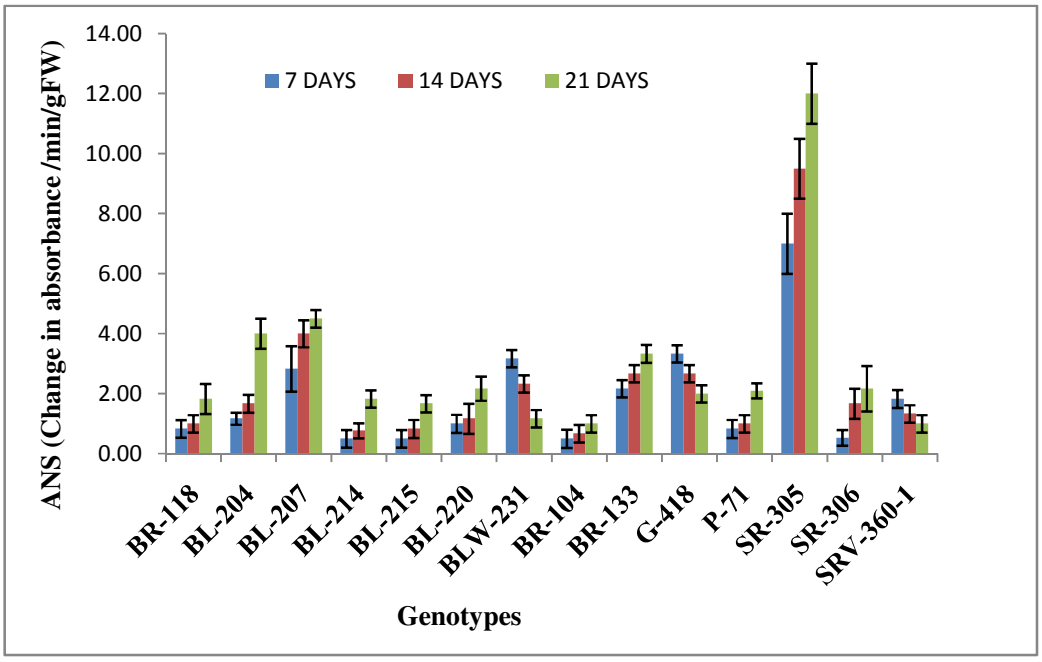


Fig 10: Anthocyanidin Synthase (ANS) activity in fruits of *Solanum melongena* L. genotypes at various days after anthesis.

Table 20: Phenylalanine ammonia lyase (PAL) activity in leaf at various stages of growth of brinjal

Name of the Genotype	Colour of the Genotype	Days After Anthesis (DAA)			MEAN
		7	14	21	
BL-207	Purple	5.20±1.13 (0.041±0.03)	29.40± 1.07 (0.590±0.12)	13.93±1.04 (0.140±0.04)	**16.18**
BL-204	Purple	2.77±1.10 (0.010±0.01)	46.57±1.10 (3.100±0.10)	22.03±1.04 (0.200±0.04)	**23.79**
BL-214	Purple	5.06±1.07 (0.040±0.01)	22.90±1.10 (0.230±0.01)	12.63±1.04 (0.120±0.04)	**13.53**
BL-215	Purple	1.53± 1.07 (0.010±0.01)	49.70±1.00 (4.117±0.01)	22.30±1.04 (0.240±0.04)	**24.51**
BL-220	Purple	5.10±1.20 (0.043±0.02)	41.70±1.00 (2.193±0.01)	27.30±1.20 (0.260±0.02)	**24.70**
BLW-231	White	5.26±1.04 (0.043±0.04)	31.73±1.07 (0.860±0.01)	9.30±1.00 (0.090±0.01)	**15.43**
BR-104	Purple	4.50± 1.20 (0.673±0.41)	38.40±1.07 (1.740±0.17)	23.70± 1.04 (0.253±0.04)	**22.20**
BR-118	Purple	3.87±0.47 (0.010±0.07)	57.37±1.04 (5.423±0.06)	20.20±0.47 (0.270±0.15)	**27.15**
BR-133	Purple	4.60±1.13 (0.020±0.03)	55.00±1.00 (5.157±0.01)	23.63±1.04 (0.257±0.01)	**27.75**
G-418	Green	22.30± 1.00 (0.240±0.01)	61.33±1.76 (6.197±0.06)	38.90±1.10 (1.923±0.10)	**40.84**
P-71	Purple	5.70±1.10 (0.044±0.01)	60.60±1.07 (6.153±0.07)	29.70± 1.00 (0.507±0.01)	**32.00**
SR-305	Purple	4.87±0.33 (0.013±0.03)	32.57±1.10 (0.097±0.01)	16.97±1.04 (0.073±0.01)	**18.13**
SR-306	Purple	4.57±0.48 (0.023±0.01)	58.60±1.07 (0.970±0.01)	12.90±1.10 (0.130±0.01)	**25.35**
SRV-360-1	White	5.80±0.30 (0.045±0.03)	18.80±1.00 (0.187±0.01)	15.07±1.07 (0.150±0.07)	**13.22**
MEAN		**8.58**	**41.59**	**19.43**	
CD (5%)		**A** = Genotypes = 0.291 **B** = DAA = 0.134 **AxB** = 0.504			

Phenylalanine ammonia lyase (PAL) activity is expressed as µmoles of cinnamic acid formed min^{-1} g^{-1} FW

Values in the parenthesis indicate specific activity (µmoles / min/ mg protein) of the enzyme

Values are mean±S.D. of the three replications

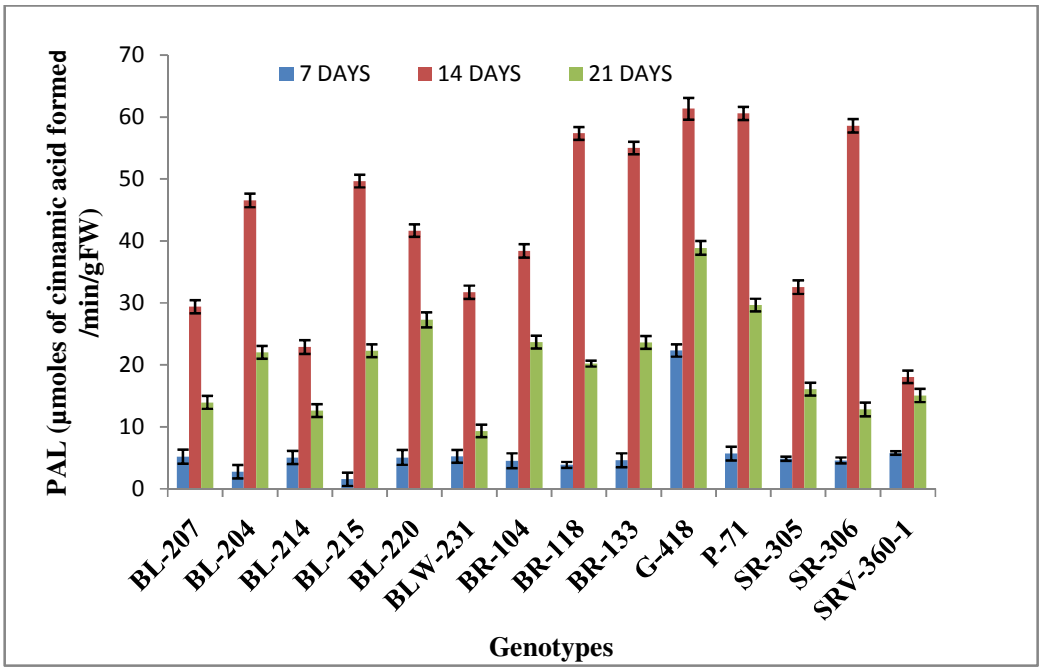


Fig 11: Phenylalanine ammonia lyase (PAL) activity in leaves of *Solanum melongena* L. genotypes at various days after anthesis.

days (Table 20, Fig 11) in genotype G-418. Genotype P-71 also showed high PAL activity at 14 days (60.60 µmoles cinnamic acid formed min^{-1} g^{-1} fresh tissue).

Fruits: The variation in PAL activity in fruits of *Solanum melongena* L. genotypes is represented in Table 21 and fig 12. The PAL activity in fruits also showed a steep increase in PAL activity till 14 days and then decrease till 21 days. The maximum PAL activity was attained in the fruits of BR-133 (65.33 µmoles cinnamic acid formed min^{-1} g^{-1} fresh tissue) at 14 days. Murtaza *et al* (2013) studied the PAL activity in six cultivars of fenugreek and found that there was a gradual increase in PAL activity from seed stage to mid stage followed by slight decrease till late growth. Our data also presented the similar pattern of PAL activity in both leaves and fruits during growth of genotypes of brinjal. The fruits have higher PAL activity (µmoles cinnamic acid formed min^{-1} g^{-1} fresh tissue) in fruits than in leaves.

4.2.3 Activity of TAL enzyme

The data in Tables 22 and 23 (Fig 13 and 14) representing the activity of TAL in leaves and fruits of *Solanum melongena* L. at various days after anthesis.

Leaves: TAL showed increase in activity from 7 days to 14 days followed by a slight decrease upto 21 days. The maximum activity was 39.63 µmoles p-coumaric acid formed min^{-1} g^{-1} fresh tissue in the genotype BL-204.

Fruits: In fruits TAL activity followed a similar trend as in leaves. TAL activity showed a steep increase in activity from 7 days to 14 days and then decrease till 21 days. The maximum

Table 21: **Phenylalanine ammonia lyase (PAL) activity in fruit at various stages of growth of brinjal.**

Name of the Genotype	Colour of the Genotype	Days After Anthesis (DAA)			MEAN
		7	14	21	
BL-207	Purple	17.30±1.00 (0.720±0.02)	33.27±1.07 (4.380±0.04)	13.93±1.04 (0.497 ±0.04)	**21.50**
BL-204	Purple	9.13±1.13 (0.177±0.01)	39.93±1.04 (8.123±0.01)	22.03±1.04 (1.087±0.01)	**23.70**
BL-214	Purple	24.30± 1.04 (0.160±0.04)	30.03±1.04 (3.093±0.01)	12.63±1.04 (0.377±0.01)	**22.32**
BL-215	Purple	7.93±1.07 (0.047±0.01)	40.90±0.30 (8.820±0.07)	22.30±1.04 (1.137±0.04)	**23.71**
BL-220	Purple	8.20±1.10 (0.097±0.01)	28.87±1.13 (2.177±0.01)	27.30±0.40 (2.213±0.01)	**21.45**
BLW-231	White	30.07±1.07 (3.123±0.01)	58.27±1.07 (17.573±0.01)	9.30±1.00 (0.190±0.06)	**32.54**
BR-104	Purple	35.03 ±1.04 (5.040±0.04)	37.00±1.00 (7.190±0.01)	23.70±1.04 (0.143±0.04)	**31.91**
BR-118	Purple	8.70±1.00 (0.100±0.07)	57.37±1.04 (16.650±0.06)	42.60±1.07 (9.030±0.07)	**36.22**
BR-133	Purple	12.30± 1.00 (0.340±0.01)	65.53±1.07 (20.477±0.04)	23.63±0.04 (0.140±0.01)	**33.15**
G-418	Green	22.97± 1.04 (1.080±0.04)	38.27±1.07 (8.057±0.07)	22.30±1.00 (1.047±0.04)	**27.84**
P-71	Purple	12.27±1.07 (0.310±0.03)	42.93±1.04 (9.103±0.01)	29.70±1.00 (3.010±0.01)	**28.30**
SR-305	Purple	22.53±0.41 (1.020±0.01)	61.73±1.07 (18.080±0.07)	16.97±1.04 (0.620±0.04)	**33.74**
SR-306	Purple	14.30±1.00 (0.510±0.12)	58.60±1.07 (17.270±0.07)	18.90±0.37 (0.733±0.01)	**30.60**
SRV-360-1	White	12.90±1.10 (0.380±0.01)	36.27±1.07 (7.253±2.50)	34.33±1.04 (5.247±0.01)	**27.83**
MEAN		**18.53**	**44.78**	**21.30**	
CD (5%)		**A** = Genotypes = 0.103 **B** = DAA = 0.047 **AxB** = 0.178			

Phenylalanine ammonia lyase (PAL) activity is expressed as µmoles of cinnamic acid formed min^{-1} g^{-1} FW

Values in the parenthesis indicate specific activity (µmoles / min/ mg protein) of the enzyme

Values are mean±S.D. of the three replications

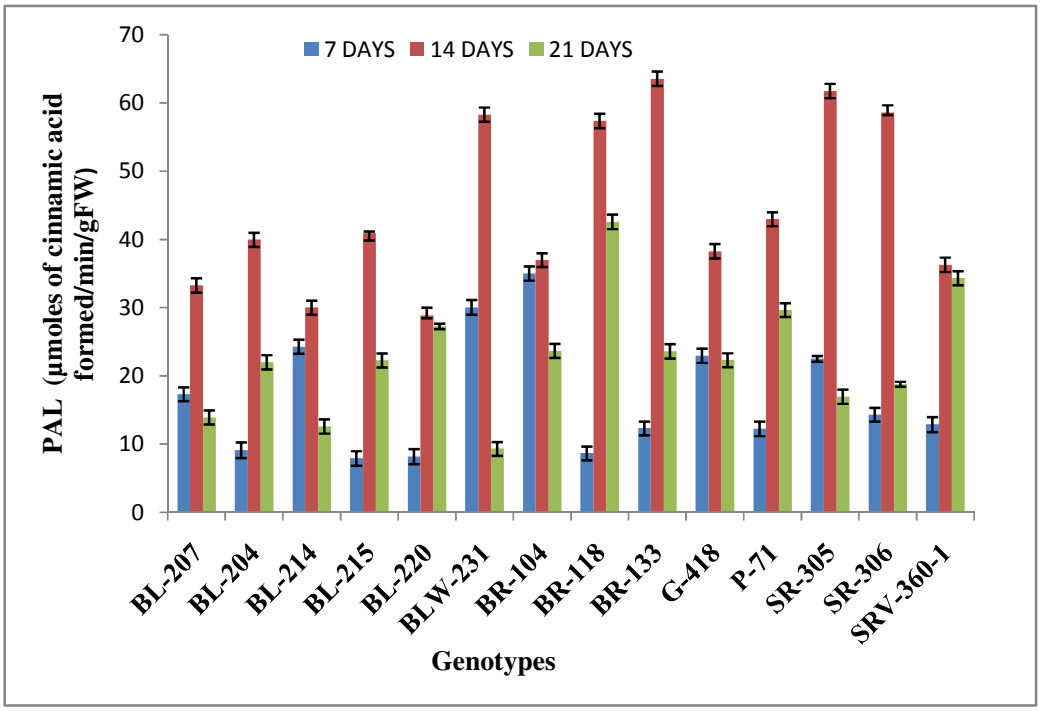


Fig 12: Phenylalanine ammonia lyase (PAL) activity in fruits of *Solanum melongena* L. genotypes at various days after anthesis.

activity attained was 41.73 µmoles p-coumaric acid formed min^{-1} g^{-1} fresh tissue in the genotype BL-214.

TAL also showed a slightly higher activity (µmoles p-coumaric acid formed min^{-1} g^{-1} fresh tissue) in fruit tissue as compared to that in leaves.

Mehta and Bhavnaryana (1981) reported that PAL and TAL enzymatic activities are highest at the growth period of 15 days of brinjal. Thereafter the activities of both enzymes decreased sharply. Our results are also in accordance with above. Similar trend was shown by Beaudoin-Eagan and Thorpe (1985) in tobacco callus culture during initiation. Rosler *et al* (1997) and Wajahatullah *et al* (2002) proposed that simultaneous activation of these enzymes depends on the species, genotype, environmental conditions and the availability of endogenous substrates. This simultaneous activation induced a strong synthesis of phenolic compounds.

Table 22: Tyrosine ammonia lyase (TAL) activity in leaf at various stages of growth of brinjal genotypes

Name of the Genotype	Colour of the Genotype	Day After Anthesis (DAA)			MEAN
		7	14	21	
BL-207	Purple	4.90±1.03 (0.087±0.03)	18.43±0.54 (0.767±0.06)	12.97±1.02 (0.340±0.02)	**12.10**
BL-204	Purple	18.63±1.03 (0.760±0.05)	39.63±1.05 (9.173±0.01)	24.87±1.05 (2.060±0.05)	**27.71**
BL-214	Purple	11.50±1.02 (0.253±0.02)	23.80±1.03 (1.163±0.03)	15.20±1.04 (0.507±0.04)	**16.83**
BL-215	Purple	4.87±1.02 (0.080±0.02)	14.27±1.04 (0.430±0.04)	13.03±0.40 (0.303±0.004)	**10.72**
BL-220	Purple	6.93±1.13 (0.080±0.03)	20.20±1.02 (1.020±0.002)	13.60±0.58 (0.330±0.08)	**13.58**
BLW-231	White	4.90±1.00 (0.080±0.01)	16.40±1.04 (0.640±0.04)	15.20±1.03 (0.500±0.03)	**12.17**
BR-104	Purple	5.70±1.03 (0.140±0.03)	34.37±0.95 (7.137±0.05)	16.03±1.04 (0.637±0.04)	**18.70**
BR-118	Purple	4.03±1.05 (0.079±0.5)	16.30±0.49 (0.603±0.09)	13.97±1.16 (0.333±0.05)	**11.43**
BR-133	Purple	6.93±0.10 (0.123±0.02)	35.70±1.00 (7.113±0.05)	17.53±1.18 (0.780±0.01)	**20.05**
G-418	Green	5.33±1.02 (0.120±0.02)	22.20±1.03 (1.070±0.03)	15.60±1.03 (0.530±0.03)	**14.38**
P-71	Purple	12.20±1.00 (0.220±0.01)	34.20±1.00 (6.090±0.01)	20.63±1.05 (1.077±0.05)	**22.34**
SR-305	Purple	10.93±1.05 (0.230±0.05)	26.00±1.02 (3.107±0.01)	24.57±0.64 (2.077±0.01)	**20.50**
SR-306	Purple	12.67±1.02 (0.293±0.01)	26.90±1.02 (3.210±0.02)	11.13±0.43 (0.210±0.03)	**16.90**
SRV-360-1	White	15.30±1.03 (0.507±0.01)	34.27±1.03 (7.150±0.03)	21.83±1.05 (1.000±0.05)	**23.80**
MEAN		**8.92**	**25.74**	**17.03**	
CD (5%)		**A** = Genotypes = 0.225	**B** = DAA = 0.104		**AxB** = 0.390

Tyrosine ammonia lyase (TAL) activity is expressed as µmoles of tyrosine formed min^{-1} g^{-1} FW

Values in the parenthesis indicate specific activity (µmoles/ min/ mg protein) of the enzyme

Values are mean±S.D. of the three replications

Table 23: Tyrosine ammonia lyase (TAL) activity in fruit at various stages of growth of brinjal genotypes

Name of the Genotype	Colour of the Genotype	Days After Anthesis (DAA)			MEAN
		7	14	21	
BL-207	Purple	11.23±1.02 (0.250±0.02)	29.50±1.00 (5.140±0.01)	19.17±1.05 (0.847±0.01)	**19.97**
BL-204	Purple	20.20±1.00 (0.857±0.01)	28.30±1.02 (4.070±0.02)	18.13±1.02 (0.750±0.02)	**22.21**
BL-214	Purple	10.30±1.00 (0.127±0.01)	41.73±0.45 (11.110±0.02)	27.10±1.02 (3.100±0.01)	**26.38**
BL-215	Purple	8.90±1.00 (0.050±0.01)	24.37±0.55 (1.490±0.05)	13.20±1.02 (0.327±0.01)	**15.49**
BL-220	Purple	13.60±0.51 (0.360±0.01)	17.13±1.03 (0.727±0.01)	16.77±1.05 (0.687±0.05)	**15.83**
BLW-231	White	7.60±1.02 (0.030±0.02)	25.33±1.05 (2.247±0.01)	19.77±1.03 (0.873±0.03)	**17.57**
BR-104	Purple	14.50±1.00 (0.420±0.05)	29.20±1.70 (5.070±0.07)	21.40±1.00 (0.900±0.10)	**21.70**
BR-118	Purple	15.43±1.03 (0.500±0.03)	23.20±1.00 (0.947±0.02)	13.23±0.88 (0.327±0.08)	**17.29**
BR-133	Purple	16.90±1.09 (0.697±0.01)	21.20±0.40 (0.900±0.04)	19.03±1.03 (0.810±0.03)	**19.04**
G-418	Green	14.90±1.03 (0.443±0.02)	40.03±1.25 (10.050±0.05)	23.90±0.65 (1.020±0.05)	**26.28**
P-71	Purple	9.40±1.02 (0.110±0.02)	25.40±1.03 (2.027±0.03)	23.03±1.05 (1.000±0.04)	**19.28**
SR-305	Purple	17.00±1.00 (0.710±0.01)	25.90±1.03 (2.570±0.05)	17.67±1.05 (0.730±0.05)	**20.19**
SR-306	Purple	10.50±0.48 (0.160±0.08)	28.90±1.00 (4.030±0.09)	18.30±1.02 (0.763±0.02)	**19.23**
SRV-360-1	White	25.83±0.74 (2.137±0.01)	46.10±1.03 (17.067±1.69)	16.50±1.00 (0.640±0.05)	**29.48**
MEAN		**15.47**	**27.57**	**19.09**	
CD (5%)		**A** = Genotypes = 0.303 **B** = DAA = 0.140 **AxB** = 0.525			

Tyrosine ammonia lyase (TAL) activity is expressed as µmoles of tyrosine formed min^{-1} g^{-1} FW

Values in the parenthesis indicate specific activity (µmoles / min/ mg protein) of the enzyme

Values are mean±S.D. of the three replications

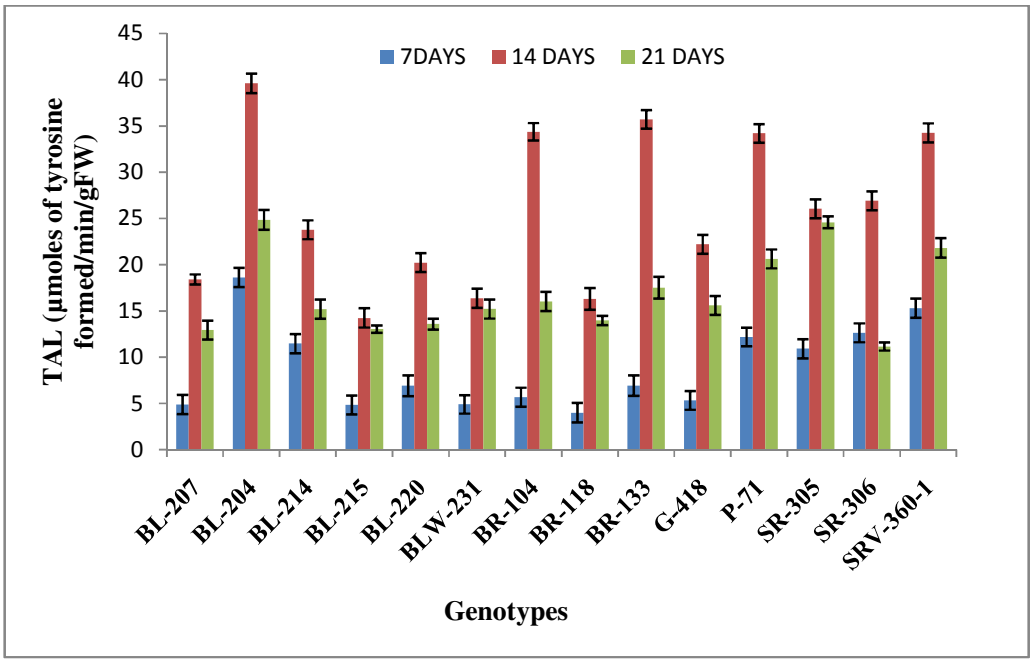


Fig 13: Tyrosine ammonia lyase (TAL) activity in leaves of *Solanum melongena* L. genotypes at various days after anthesis.

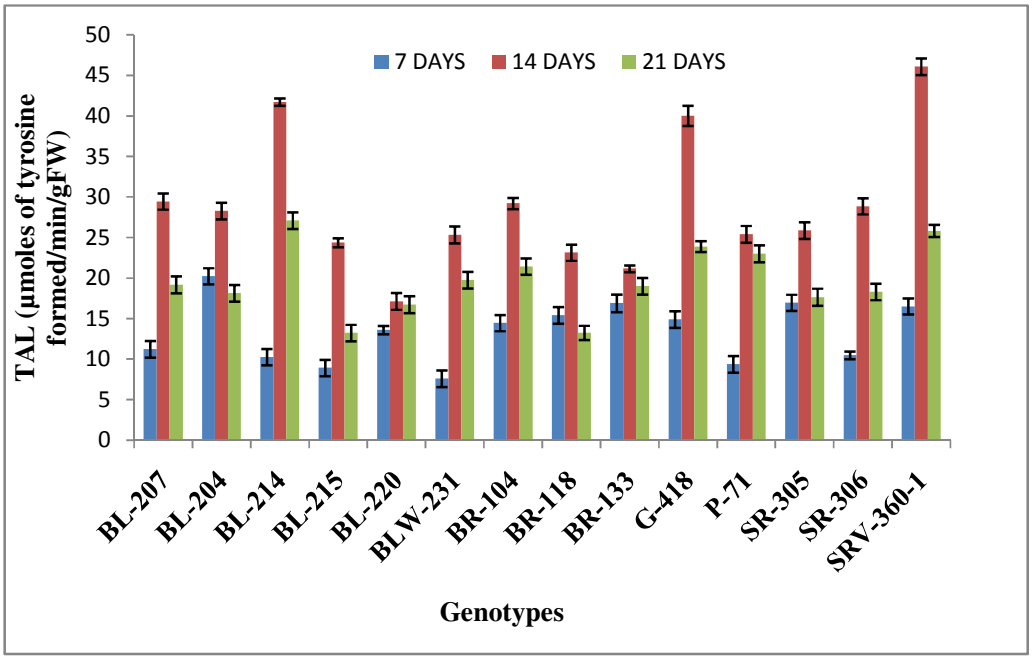


Fig 14: Tyrosine ammonia lyase (TAL) activity in fruits of *Solanum melongena* L. genotypes at various days after anthesis.

CHAPTER V

SUMMARY

Brinjal (*Solanum melongena* L.) is a tropical and subtropical crop of warm areas. Brinjal belongs to the Solanaceae family which also includes crops such as potato, sweet pepper and tomato. The crop is adapted to different agro-climatic regions and can be grown throughout the year. India is the second largest producer of brinjal whereas China is at first rank. In India, brinjal occupies an area of 7.2 million ha with a production of 134.4 million MT. In Punjab production of brinjal is 81.27 thousand MT in 3.8 thousand ha area. Brinjal is also known as eggplant due to its resemblance with hen's egg. It is commonly known as "Baingan" in Hindi. It is used to prepare many dishes and pickles throughout the world.

According to USDA Nutrient Data Base, 100 g of eggplant contains energy (24 kcal), carbohydrates (5.7 g), fat (0.19 g), protein (1.01 g), thiamine (0.039 mg), riboflavin (0.037 mg), niacin (0.649 mg), panthenothenic acid (0.281 mg), vitamin B6 (0.084 mg), folate (0.22 mg), vitamin C (2.2 mg), calcium (9 mg), iron (0.24 mg), magnesium (14 mg), manganese (0.25 mg), phosphorus (25 mg), potassium (230 mg) and zinc (0.16 mg). In addition to vitamins and minerals, brinjal also contain phytonutrients which include phenolic compounds and flavonoids. Anthocyanins constitute an important class of flavonoids which give pink, red and purple colours to flowers, fruits and other vegetative tissues of plant. Anthocyanins also have an important role in UV protection, defence against pathogens and pests in plants. Along with plant protection, anthocyanins have human health promoting effects. They have role in enhancing the right acuteness, anti-ulcer and anti-mutagenic activity. Chlorogenic acid, main phenolic compound found in brinjal also contributes to antimutagenic, antimicrobial, anti-low density lipoprotein and antiviral activities. Because of these medicinal properties brinjal can be used as "Nutraceuticals". Anthocyanins are also good alternative to synthetic colours, thus can be used as natural colorants. Brinjal skin colour may be purple, green, white or many other colours. This is related to the anthocyanin content present in brinjal skin.

The present investigation was undertaken to know the content of anthocyanins in peel, flesh and whole fruit. Other biochemical constituents such as sugars, total phenols, ODHs, flavonols along with the activities of enzymes ANS, PAL and TAL involved in flavonoids biosynthesis pathway were also studied.

Fifty genotypes of brinjal having different fruit colour, shape and size were evaluated for analysis of anthocyanins, total sugars, total phenols, ODHs and flavonols in two trials. Anthocyanin content was estimated from fresh tissue while total sugars, total phenols, ODHs and flavonols were estimated from dried samples in 2012 and 2013.

The anthocyanin content in peel was in the range of 0.04-113.93 mg/100g in 2012

and 0.05-109.02 mg/100g in 2013. The highest anthocyanin content in peel was found in SR-312. The genotypes exhibiting anthocyanin content > 90mg/100g in peel were BL-201, BL-202, BL-204, BL-207, BL-214, BL-215, BL-219, BL-220, BR-104, BR-118, BR-133, MR-319, MR-320, P-71, SR-301, SR-302, SR-303, SR-304, SR-305, SR-306, SR-307, SR-309, SR-310, SR-311, SR-312, SR-313 and SR-318.

The anthocyanin content in flesh part of brinjal fruit was ranged from 0.01 to 9.89 mg/100g in 2012 and from 0.03 to 6.84 mg/100g in 2013. The genotype having highest anthocyanin content in flesh was SR-308. The genotypes exhibiting anthocyanin content > 2mg/100g in flesh were SR-308, SR-304, SR-306, SR-312, SR-318, BRG-224 and BLEND-11-WR-1.

The whole fruit have anthocyanin content in the range of 0.55-88.24 mg/100g in 2012 and 1.87-88.91 mg/100g in 2013. The highest content was found in SR-303. The genotypes exhibiting anthocyanin content > 60 mg/100g in whole fruit were BR-118, SR-303, SR-318.

Brinjal is known to have low total soluble sugar content. The content varied in the range 0.53-24.77% in 2012 and 0.71-20.36% in 2013. The genotypes showed sugar content in the range 5-10% were BL-204, BL-207, BL-214, BLW-231, BLEND-11-WR-2, SR-308, BR-133, G-405, G-411, BRG-111, MR-320, SR-301, SR-302, SR-303, SR-305, SR-311, SR-318, BR-104, W-230-42-45-1.

Total phenols were found in the range of 70.21-296.37 mg/100g in 2012 and 75.16-298.63 mg/100g. The genotypes exhibiting total phenol content >200 mg/100g were G-412, G-414 and G-415.

ODH content in investigated genotypes of brinjal in the range of 3.41-93.00 mg/100g in 2012 and 3.69-98.72 mg/100g in 2013. Regarding ODH content, the genotypes P-71, G-407, G-415 and G-418 exhibited higher ODH content i.e > 60 mg/100g. The highest ODH content was found in P-71.

The content of flavonols was found in the range from 25.17-847.78 mg/100g in 2012 and 21.38-835.86 mg/100g in 2013. MR-320 and BLEND-WR-11-2 exhibited high flavonol content (> 600 mg/100g).

From these observations, it was concluded that the genotypes have highest content of anthocyanins in peel tissue followed by whole fruit and flesh tissue. The genotypes having fruits of purple colour have high anthocyanin content than fruits of other colours. Genotypes having green fruits have high total phenol, ODH content and low sugar content. The genotypes bearing white fruit have high flavonol content. A positive correlation was found between anthocyanins, flavonols in peel and whole fruit. Therefore, it implies that peel accumulated large amount of anthocyanins and phenolic compounds than flesh part. Ten genotypes were selected having best combination of all characteristics. The genotypes were BR-118, MR-319, MR-320, SR-303, SR-304, SR-306, SR-310, SR-311, SR-312 and G-411.

After analysis of brinjal genotypes for anthocyanins and phenolic compounds 14 genotypes viz. BR-118, BL-204, BL-207, BL-214, BL-215, BL-220, BL-231, BR-104, BR-133, G-418, P-71, SR-305, SR-306, SRV-360-1 were selected for assay of three enzymes ANS, PAL and TAL. The sampling was done at 7, 14 and 21 day after anthesis (DAA). The enzymes were extracted from leaf and fruit tissues in phosphate buffer and activity was measured from fresh tissue. The three enzymes have different patterns of activity during growth of brinjal genotypes.

ANS had highest activity at 7 days and then decreased up to 21 days in leaves while there was increase in activity in fruit tissue with purple colour during growth. Decrease in ANS activity was recorded in genotypes with green and white fruits. The genotype exhibiting highest activity of ANS in leaves was BL-204 and in fruits SR-305. From this it was concluded that ANS activity varied in accordance with anthocyanin accumulation in tissue.

PAL and TAL have similar pattern of activities. The activities of both enzymes increase from 7 days, were highest at 14 days and then decreased upto 21 days during growth. The PAL enzyme had highest activity in genotype G-418 in leaves while in fruits genotype BR-133 exhibited the highest activity. Maximum activity of TAL enzyme was exhibited in BL-204 in leaves and BL-214 in case of fruits. According to literature review activities of both enzymes are related to synthesis of phenolic compounds so it was found that the genotypes having high activity of PAL and TAL also exhibited high content of phenolic compounds.

From this study, it was concluded that brinjal is rich source of anthocyanins and phenolic compounds. The information regarding the association of specific biochemical characters within different genotypes can be exploited by the breeder to produce the model genotype having many characters which can be exploited by industry to produce nutraceuticals as well as natural colorant.

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VITA

Name of the student	**:**	Ankita Kumari
Father's name	**:**	Mr. Amrik Singh
Mother's name	**:**	Mrs. Sunita kumari
Nationality	**:**	Indian
Date of birth	**:**	14th January, 1993
Permanent home address	**:**	Vill. Kariyal, P.O. Rappar, Gangath, Teh. Nurpur, Distt. Kangra, Himachal Pradesh-176204

EDUCATIONAL QUALIFICATION

Bachelor degree	**:**	B.Sc. (Hons.) Biotechnology
University and year of award	**:**	Himachal Pradesh University, Summer Hill, Shimla, 2012
%age of marks	**:**	75.50 %
Master's degree	**:**	M.Sc. (Biochemistry)
University and year of award	**:**	Punjab Agricultural University, Ludhiana 2014
OCPA	**:**	7.92/10.00
Title of Master's thesis	**:**	A study on anthocyanins and other biochemical constituents in different plant parts of brinjal