"BIOCHEMICAL STUDIES ON THE DEVELOPMENT OF ARIL BROWNING IN POMEGRANATE"

HEMLATA SINGH

PAK 9243

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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HEMLATA SINGH

PAK 9243

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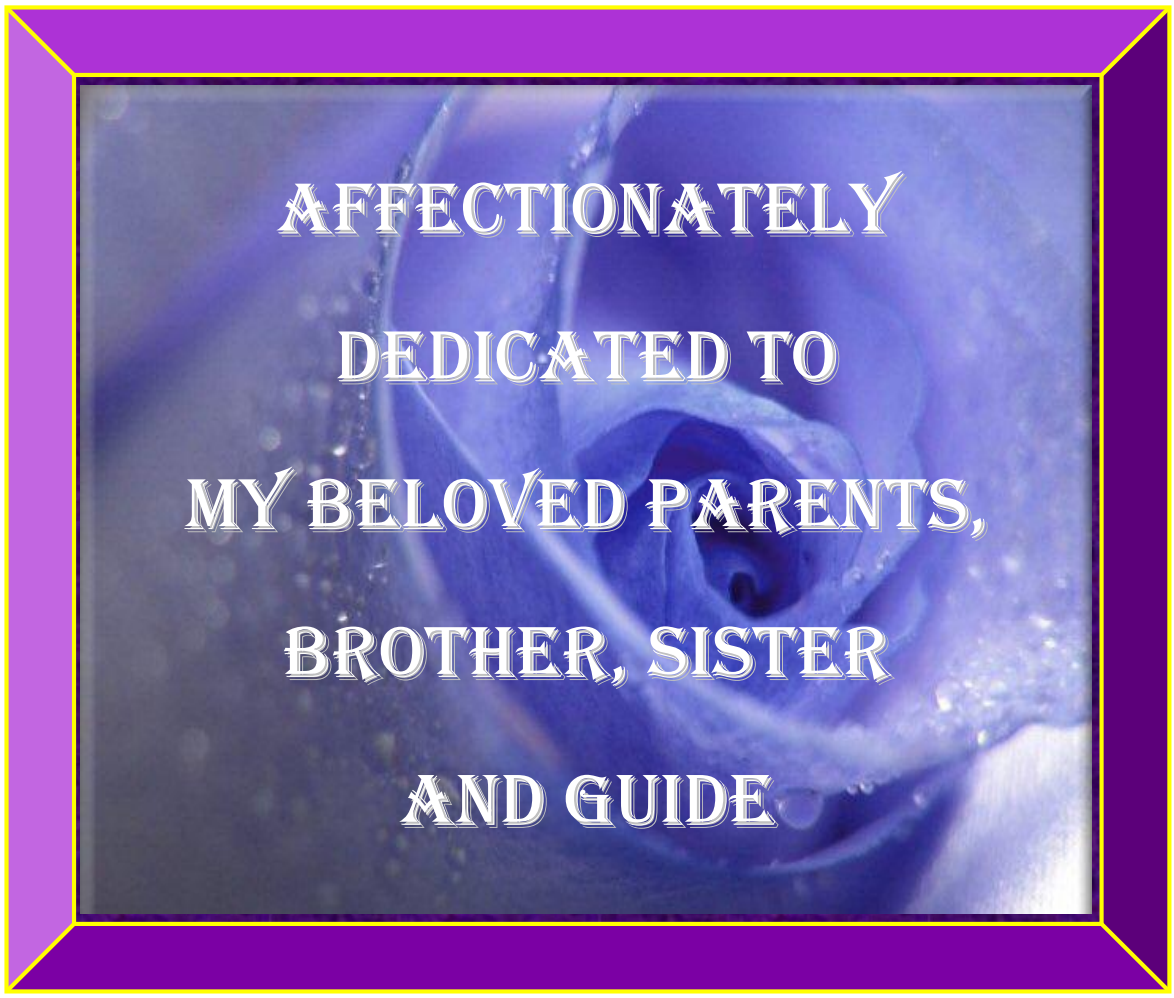
AFFECTIONATELY
DEDICATED TO
MY BELOVED PARENTS,
BROTHER, SISTER
AND GUIDE

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BANGALORE 560065

CERTIFICATE

This is to certify that the thesis entitled "**Biochemical studies on the development of Aril browning in pomegranate"** submitted by **Ms. HEMLATA SINGH, ID No. PAK 9243,** for the award of degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT BIOCHEMISTRY** to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by her during the period of her study under my guidance and supervision and that no part of this thesis has been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
July, 2011

Dr. S. SHIVASHANKAR
Principal scientist and head
Div of Plant Physiology and Biochemistry
IIHR Hesaragatta Lake Post, Bengaluru-89

Approved by:

Chairman : _____
(S. SHIVASHANKAR)

Co-Chairman : _____
(P.H. RAMANJINI GOWDA)

Members : **1.** _____
(G.K. MUKUND)

2. _____
(S.H.JALIKOP)

3. _____
(S.K.THIMMAIAH)

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"BIOCHEMICAL STUDIES ON THE DEVELOPMENT OF ARIL BROWNING IN POMEGRANATE"

Abstract

Aril browning (AB) in pomegranate is a physiological disorder free of external symptoms. Browning of aril starts with a dark dot on the aril and spreads further to the entire aril. The incidence is at first observed at 50% fruit maturity near the calyx end just under the skin. Present studies showed that AB incidence was higher in panicles with increasing number of fruits and in fruits located on the lateral shoots as compared to those on main shoots. Fruits exposed to sun showed lesser incidence. AB incidence also increased with fruit maturity. Biochemical studies revealed that sugars, TSS, starch and pH were higher in AB affected aril as compared to healthy arils whereas anthocyanin, polyphenols, titrable acidity, protein and ascorbic acid were less in AB affected aril. Enzyme activities like amylase, total dehydrogenase activity in seed were reduced in seed of AB affected aril compared to healthy whereas enzyme activity like polyphenol oxidase was more in seed of AB affected aril as compared to seed of healthy aril. Healthy arils showed higher moisture content and the seed higher percentage and faster rate of germination as compared to seed of AB affected aril, revealing that seed of AB affected aril had lost moisture leading to reduction in seed viability. Field experiments with growth regulators showed that GA_3 treatment reduced incidence of AB and PBZ treatment increased the incidence of browning as compared to control. These findings indicated that the development of AB in pomegranate is a result of combination of many factors like interfruit competition, biochemical and physiological changes in aril during fruit growth.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

I INTRODUCTION

The pomegranate (*Punica granatum* L.) is native to the region extending from Northern India to Iran. Since ancient times, the pomegranate was cultivated throughout the Mediterranean region of Asia, Africa and Europe. The pomegranate tree is a fruit bearing deciduous shrub or small tree between five and eight meters tall. The name of pomegranate appears to be from latin word *pomum* meaning apple and *granatus*, which means seeded. The genus name *Punica* is named after the people of the Phoenicians who were active in expanding its cultivation. Punica was also the Roman name for the city of Carthage founded by the Phoenicians, from where the best pomegranates came to Italy (Nath *et al.,* 2008).

The tree belongs to the family *Punicaceae*. The *Punicaceae* family consists of only one genus and two species viz *Punica granatum L.* and *Punica protopunica* L. This last species is endemic to the island of Socotra. The pomegranate is rounded shrub or small tree that can grow to a height of 20 or 30 feet (6 to 9 meters), but more typically to 12 or 16 feet (4 to 5 meters) in height. The pomegranate is highly branched, with stiff, angular and spiny branches. The trunk is covered by a red-brown bark, which later becomes grey. Fruit is nearly round, 2 to 5 inches in diameter and is crowned at the base by the prominent calyx, the crown. Fruit weight ranges between 200 and 500 grams. The tough, leathery skin or rind is typically yellow overlaid with light or deep pink or rich red. Phenolic compounds determine the colour of the pomegranate. The interior is separated by membranous walls and white, spongy, bitter tissue into compartments packed with sacs filled with sweet acid, juicy, red, pink or whitish pulp or aril. In each sac, there is one angular, soft or hard seed. Each fruit contains hundreds of seeds.

The edible part of the fruit is the arils which constitute 52% by weight of fruit, comprising 78% juice and 22% seeds. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins and it is also reported to be a rich source of antioxidants. Pomegranate is a high value crop and its entire tree is of great economic importance. Apart from the demand for fresh fruits and juice, the processed products like wine and candy are also gaining importance in world trade. All parts of pomegranate tree have great therapeutic value and are used in leather and dying industry.

The fast increase in demand for the fruit in the international market has widened the scope for earning higher dividends from this crop. Profits up to Rs.1.5 Lakh ha/annum have been demonstrated by some growers. It is, therefore, a highly remunerative crop for replacing subsistence farming and thus alleviating poverty levels, particularly in regions such as Karnataka and Maharashtra. It is an ideal crop for the sustainability of small holdings, as pomegranate is well suited to the topography and agro-climate of arid and semi-arid regions. In addition, it provides nutritional security, has high potential to develop wastelands widely available in the region and an ideal crop for diversification. Moreover, it can make higher contribution to GDP with a small area. There has been a steady increase in area and production of pomegranate in the country. It is estimated that by the year 2025, the area under pomegranate is projected to increase to 7.5 lakhs ha, from 1.25 lakhs ha at present. Consequently production is expected to increase by 10 folds and export by nearly seven folds by the year 2025.

Table 1. Nutritive value of pomegranate

Food value per 100 g of edible portion	
Calories	63-78
Moisture	72.6-86.4 g
Protein	0.05-1.6 g
Fat	≤ 0.9 g
Carbohydrates	15.4-19.6 g
Fibre	3.4-5.0 g
Ash	0.36-0.73 g
Calcium	3-12 mg
Phosphorus	8-37 mg
Iron	0.3-1.2 mg
Sodium	3 mg
Potassium	259 mg
Carotene	None to Trace
Thiamine	3 µg
Riboflavin	12-30 µg
Niacin	18-30 µg
Ascorbic Acid	4-4.2 mg
Citric Acid	0.46-3.6 mg
Boric Acid	5 µg

Source:-UASD National Nutrition Database

In value terms grapes and mango exports earn the maximum foreign exchange for India. However, there is immense potential to diversify this product basket, and one such fruit is pomegranate. Major pomegranate producing states in India are Maharashtra, Karnataka, Andhra Pradesh, Gujarat, Rajasthan, Tamil Nadu and Himachal Pradesh. Pomegranate is an important fruit crop of Maharashtra. It is cultivated in an area of 969,000 ha with a total production of 555.5 million tonnes producing about 67.7 % (Indian Horticulture Database, 2009-2010) of the total Indian production, thereby leading in Pomegranate production in the country. Solapur, Nasik, Sangli, Ahmednagar, Pune have the maximum area under pomegranate in Maharashtra and Bellary, Bijapur and Chitradurga have in Karnataka state which contribute about 16.9% of total production.

At the global level, Iran is the world's largest producer and exporter of pomegranates with an estimated annual production of 670,000 tons, In addition to Iran, other countries including India, Turkey, Spain, Tunisia, Morocco, Afghanistan, China, Greece, Japan, France, Armenia, Cyprus, Egypt, Italy and Palestine also cultivate this crop. Spain exports pomegranates from September to December months which decrease from January onwards. Major exports from Spain are to European Union. Iran exports are mainly to Gulf countries and supplies are at peak during October-December and it decreases from January onwards. In India, its peak production is during December-March and continues up to April-June. Thus, India can export pomegranates from February to June months when there will be no competition from Spain.

In order to meet the growing demand for fruit in both domestic and export market, there is need to maintain high quality of fruits. The high incidence of the physiological disorder called ‘aril browning (AB)’ or ‘aril blackening’ (internal breakdown of arils) has threatened the popularity of

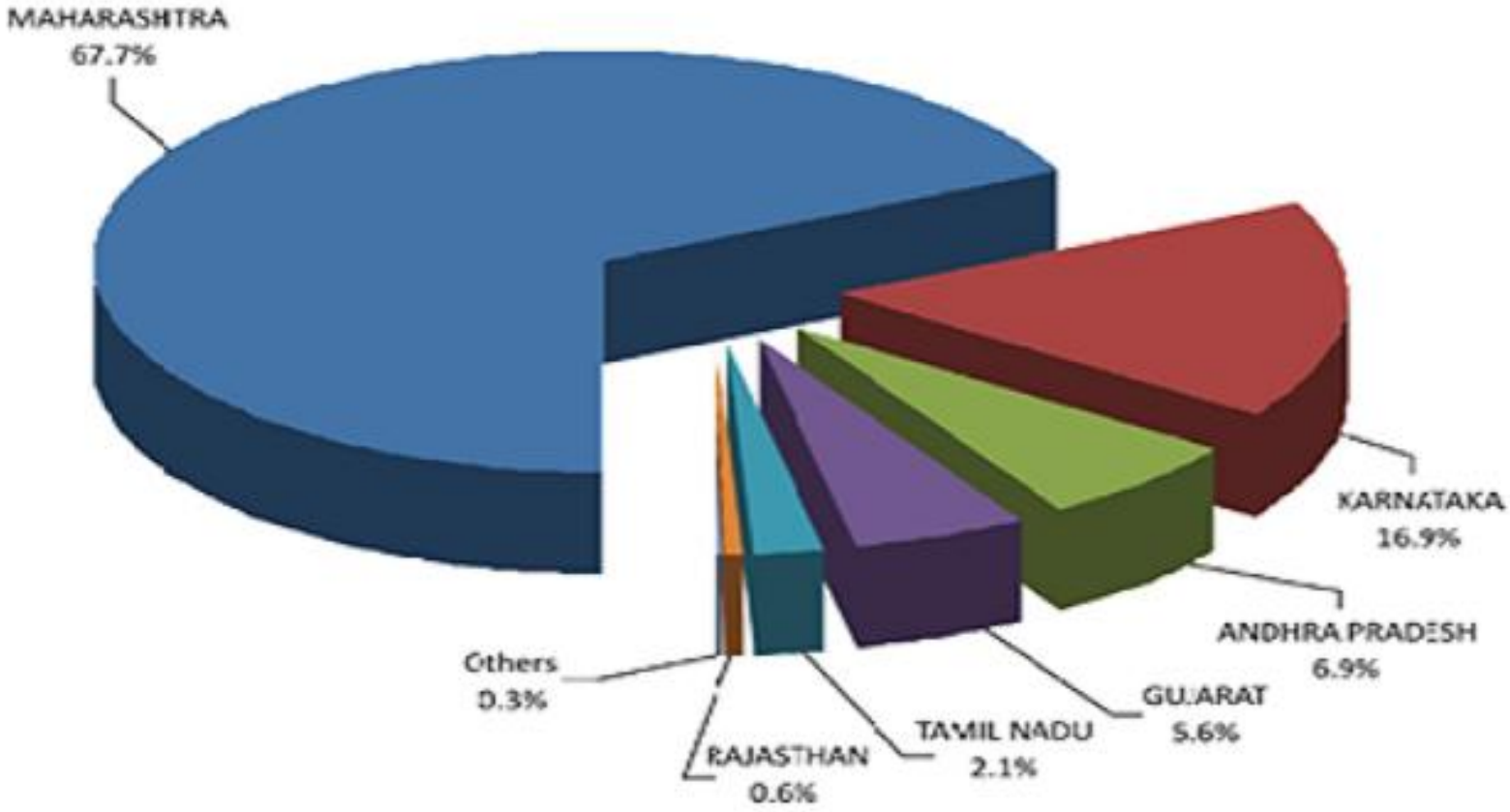


Fig. 1. Production of pomegranate in different states of India during 2009-2010 (Indian Horticulture Database, 2010)

pomegranate fruit. The problem has confronted growers, processors, consumers, exporters and researchers, alike.

As the exact cause of this malady is not known, remedial measures are difficult to advocate. If the problem of aril browning remains unresolved it could affect the consumption and export of pomegranate fruit. So, there is an urgent need to understand the mechanism of aril browning in pomegranate. Therefore the present study was undertaken with the following objectives,

1. Effect of fruit location, fruit maturity and fruit number on biochemical parameters and the incidence of aril browning.

2. Dehydrogenase activity in seed and its relation to incidence of aril browning.

3. To study the biochemical changes during the formation of aril browning and the effect of applied plant growth regulators on the incidence of browning in pomegranate.

Review of Literature

II REVIEW OF LITERATURE

In this chapter, a summary of available literature on the importance of competition among different sinks of plant, role of seed and growth hormone, effect of pre harvest factor, nutritional aspect and biochemical composition in seed and juice in relation to development physiological disorder, aril browning of pomegranate is presented.

Physiological disorders are distinguished from other disorders in that they are not caused by living organisms (viruses, bacteria, fungi insects etc), but are caused by non-living, abiotic situations which cause a deviation from normal growth. They are physical or chemical changes in a plant from what is normal. Some non-infectious disorders are easy to identify, but others are more difficult. Most of them are not reversible once they have occurred. To help in identifying physiological disorders it is important to know that:

- Physiological disorders are often caused by the lack or excess of some factor that supports life or by the presence of some factor that interferes with life.
- Physiological disorders can affect plants in all stages of their lives.
- They occur with the absence of infectious agents therefore cannot be transmitted.
- Dealing with physiological disorders often means dealing with the consequences from a past event.
- Generally there is a clear cut differences in physical, structural and biochemical parameter between healthy and damaged tissue.
- Physiological disorders are serious in themselves but often serve as the 'open door' for pathogens to enter.

Most of the fruit crops are affected by the physiological disorders; physiological disorders exert a major impact on fruit quality and consequently marketability. Some major fruit disorders are listed in Table 2.

Table 2. Physiological disorders in major fruit crops

Crop	**Disorder**	**Description**	**References**
Mango	Spongy tissue	Flesh generally paler in colour; spongy with or without air pocket and off-flavours	Subramanayam *et al.* (1971), Joshi and Roy (1985)
Mango	Fruit pitting	Development of some sunken pits on fruit peel	Sharma *et al* (2009)
Avocado	Vascular browning, pulp spot, grey pulp	Browning of pulp occur	Koen *et al* (1990)
Apple	Internal browning	Brown discoloration in the flesh, usually originating in or near core	Grant and Mitcham (1995)
Banana	Senescent spotting	Appearance of superficial brown spots	Ketsa Saochol (2000)
Kiwi fruits	Physiological pitting	Sunken pits are formed on the upper side of skin	Ferguson *et al* (2003)
Stone fruit	Internal breakdown	Browning of flesh, lack of juiciness due to leatheriness, red pigment accumulation	Crisosto *et al* (1995)

Major physiological disorders of pomegranate include cracking of fruit, husk scald, chilling injury and aril browning (Nath *et al*, 2008). Fruit cracking is a serious problem of pomegranate. It is due to boron deficiency in young fruits while in developed fruits it may be caused due to extreme variations in day and night temperatures, at the time of fruit ripening. If the soils become too dry followed by heavy irrigation or rains, cracking may occur. Delay in harvesting of fully ripened fruits for a long time or severe attack of pest and disease also leads to cracking of the fruits (Kumar, 1990). Husk scald is post harvest physiological disorder. Its symptoms include brown superficial discoloration restricted to the husk. Husk scald development occurs may be due to phenolic oxidation. Late harvested fruit is less susceptible than earlier harvested fruit. Scald development may be delayed by storing at low temperature 2°C (Defilippi *et al.*, 2006). Chilling injury is also post harvest disorder. Fruits are susceptible to chilling injury if stored longer than one month at temperatures between their freezing point -3°C (26.6°F) and 5°C (41°F).When such fruits are transferred to higher temperatures, respiration and ethylene production rates increase and other chilling injury symptoms appear. External symptoms include brown discoloration (scald) of the skin, pitting, and increased susceptibility to decay. Internal symptoms include brown discoloration of the white segments separating the arils and pale color (loss of red color) of the arils. External and internal browning is related to oxidation of phenolics by polypherol oxidase. Storage in a 2% oxygen atmosphere at temperatures below 5°C (41°F) reduces severity of chilling injury symptoms.

Aril browning in pomegranate is a physiological disorder wherein the brown flattened and soft arils are noticed when fruit is cut open. Externally one cannot make out whether a fruit is affected with aril browning or not. The browning of aril starts with a dark dot on the aril and later on spreads to the entire aril and many of them have a streaked

appearance due to fine white lines radiating from the seeds. Affected arils are soft, light creamy – brown to dark blackish – brown, deformed and possess unacceptable off-flavour and unfit for consumption and exhibit poor dessert quality. The aril browning is more prevalent in cv. Ganesh, though other cultivars are also affected with aril browning to some extent. Blackening of arils has been a serious threat to the pomegranate industry.

A review of literature shows that not much work has been carried out on this physiological disorder of pomegranate either in India or abroad. So, there is not much information available on this malady of pomegranate, the literature available on physiological disorder of other fruits like bitter pit in apples, cork spot in pears, mesocarp discoloration in avocados and spongy tissue in mango have been included for the review and is presented under the following headings :-

2.1. Preharvest factors affecting physiological disorder of fruit

    2.1.1. Horticultural aspects

    2.1.2. Ecological aspects

    2.1.3. Nutritional aspects

2.2. Inter-fruit completion for water and nutrients and its effect on the fruit disorder.

2.3. Plant growth regulator and its effect on development of disorder.

2.4. Significance of seed in fruit disorder.

2.5. Biochemical changes in fruits in relation to development of physiological disorder.

2.1. Preharvest factors affecting physiological disorder of fruit

Development of disorder of fruit depends on a range of preharvest factors. Preharvest factors can be divided into horticultural aspect which includes varietal response, stage of maturity at harvest and size and weight of fruit at harvest, ecological aspects which includes seasonal characteristics (temperature during growth, rainfall), orchard characteristics (the position of the fruit on the tree, geographical position and variety) and nutritional aspect.

2.1.1. Horticultural aspect

A. Varietal response

Desai (1989) observed that P-23 variety of pomegranate had low percentage of affected (aril browned) fruits than cv. Ganesh. Shete (1998) studied the incidence and intensity of this malady in Mrig and Ambia bahars in different varieties. He observed maximum incidence (100%) in cv. G-137 and Jalore Seedless from 105 days onward during Mrig bahar. Among all the cultivars, the cv. Mridula showed minimum incidence (50%) at 120 days after fruit set. During Ambia bahar, the per cent incidence was 20 to 100 % in cv. G-137, Gul-E-Shah Red, Jalore Seedless and Mridula. Wasker *et al.* (2003) reported that the 'Bhagwa' variety is free from blackening of arils even in case of late harvesting of fruits.

B. Stage of maturity at harvest

According to Lau (1998) there is strong harvest date effect on incidence, in common with many apple storage disorders, with later harvested fruits being more susceptible.

Under commercial condition, both maturity and size were found to vary considerably among fruit lots (Bramlage *et al.*, 1985). There was a

positive correlation between fruit maturity and occurrence of senescent breakdown and apple scald (Anet *et al.*, 1974, Lidster *et al.,* 1975).

Spongy tissue was first observed by Cheema and Dani (1934) when examining first picked fruits at 'C' stage of (full) maturity. They noticed that early picked fruits escaped the incidence of disorder to some extent, and the tendency to develop internal breakdown was more towards the end of the season. Other workers have also noted that late harvesting renders the fruit more susceptible to spongy tissue (Joshi, 1975, and Katrodia, 1979). But in early harvested fruits the quality was inferior (Rangwala, 1975, Patkar, 1978, Krishnamurthy, 1980).

The incidence of spongy tissue in Alphonso mango increased with advancing maturity (Amin *et al.*, 1974). Subramanyan *et al.*, (1971) observed that fruits with breakdown were at optimum stage of maturity. Limaye *et al.*, (1976) studied the occurrence of spongy tissue in relation to stage of maturity at harvest. They found that a low proportion (10.5%) of fruits were affected by spongy tissue when harvested at 'A' stage (12 Anna), and the maximum incidence (87.6%) was observed in fruits harvested at 'D' (tree ripe) stage. If the fruits were harvested at 'B' stage of maturity (14 Anna), the occurrence of spongy tissue was considerably low (23.33%) and the fruit quality of ripe fruits were less affected than those harvested at the fully ripe stage. Similar observation was made by Joshi (1975).

Delayed harvesting of fruit beyond 150 days might be the one of the possible reasons of browning of aril in pomegranate as reported by Khodade (1987). He further noticed that browning of aril in cv.G-137 was initiated at 150 days stage of maturity and the intensity of browning of arils increased at 158 and 165 days stages. Desai (1989) observed this malady in cv. Ganesh at 90 at 120 days stages where as it was evident at 160 days in cv. P-23.The malady in cv. Ganesh -137 was evident at

105 days during Mrig and Ambia bahars as observed by Shete (1998). He further observed that the cultivar Jalore Seedless and Mridula showed the malady at 135 days after fruit set during Ambia bahar and that in cultivar Gul-E-Shah Red, it was at 150 days. The malady in cv. Jalore Seedless and cv. Mridula was evidenced at 105 and 120 days, respectively after fruit set in Mrig bahar.

C. Size and weight of fruit at harvest

It has been reported that the occurrence of bitter pit in apple is related to the fruit size within the same tree, larger fruit being affected more than smaller fruit (Martin, 1954).

Though Khodade (1987) reported that incidence of blackening of pomegranate arils increasing with increase in fruit weight from 150 to 300g (26.6% to 60%) but Desai (1989) and Shete (1998) reported that the physical parameters like weight, volume and size of fruit did not show any relationship with the malady. Further, specific gravity of fruit also did not show any relationship with blackening as observed by Desai (1989).

2.1.2. Ecological aspects

A. Season of harvest

Seasonal characteristics like temperature during growth, rainfall are also very important preharvest factors. 'Conference' pears grown in warm growing areas are less susceptible to browning disorder than pears grown in the cold areas (Magness *et al.*, 1929 and Zerbini *et al.*, 2002).

Observations on 'Fuji' fruit in the UAS suggest that there is greater incidence after a cooler growing season (Fallahi *et al.,* 1997). A type of watercore can be related to exposure of fruit to high temperature on the tree, before fruit maturation (Faust *et al.*, 1969). Even in temperate

regions , apple fruits flesh temperatures under direct sunlight can reach in excess of 40^{o}C (Ferguson *et al.*,1989).Frequent exposure of apple fruit to such temperature will result in damage such as sunburn (Bergh *et al.,* 1980) and loss of texture (Tustin *et al.*,1993), as well as increase in disorders such as watercore.

Translucence a disorder in pineapple has been related to high nitrogen, and high radiation, temperature and rainfall during growth (Soler, 1994; Paull and Reyes, 1996). High rainfall and the consequence on fruit growth also increases incidence of skin cracking disorders, such as found in cherries (Sekse, 1995) and apples (Opara *et al.*, 1997).

Shete (1998) observed that there is difference in occurrence of aril browning of pomegranate malady during different seasons. He observed that during Ambia bahar this malady was more than Mrig bahar.

B. Location

'Conference' pears grown in the Mediterranean area are less susceptible to browning disorders than pears grown in the northwest of Europe (Zerbini *et al.*, 2002a) and 'Bartlett' pears from cool growing district (Magness *et al.*, 1929).

Desai (1989) reported that the pomegranate fruit located on southern side of tree showed higher degree of malady than that of fruits from centre of tree.

2.1.3. Nutritional aspects

As a method of control, preharvest calcium dips increased the fruit calcium, as well as reduced the occurrence of spongy tissue in Alphanso mango. Calcium chloride dips (0.5 To 2 %) were more effective than calcium nitrate dips (Gunjate *et al.*, 1979b). Katrodia (1979) studied the effectiveness of foliar sprays as well as dip treatments with $CaCl_2$

solution; calcium chloride spray or dipping had no direct effect on spongy tissue development. Dipping fruits in high (two and three per cent) concentration of $CaCl_2$ caused blackening and roughening of the fruit skin. Preharvest sprays of calcium (5000ppm) either alone, or in combination with postharvest dip treatments (5000ppm $CaCl_2$) to Alphanso mango trees neither reduced the incidence of internal breakdown, nor enhanced the calcium level of the fruits (Krishnamurthy, 1981).

Zhon Honji *et al.* (1981) recommended that $Ca(NO_3)_2$ sprays (5%) at three and five weeks after flowering and again eight and ten weeks before harvest, reduced the water core incidence. Regular calcium sprays in the orchard were found to raise the Ca level and overcome the hormone induced (GA_3 + 2,4,5,TP) lower Ca concentrations in fruits (Jackson *et al.*,1982).

Foliar sprays of calcium to apple tree have been widely practiced in the control of physiological disorders. Such foliar spray with calcium salt are the most direct way of ensuring adequate fruit calcium levels during growth. Initially, calcium nitrate was the recommended source, but tests with many other calcium salts have led to its wide spread replacement by calcium chloride (Garman and Mathis, 1956). Bitter pit – a physiological disorder of apples, can be controlled partly and sometimes entirely by spraying with solution of calcium nitrate or calcium chloride during the growing season (Van Goor, 1971; Naumann, 1974).

Calcium imbalance is the cause of bitter pit, flesh browning and softness in the apple cultivar Greenstein. 58.2 % of the fruits were subjected to bitter pit when K: Ca ratio was 35:9.Calcium Chloride sprays were more effective than calcium nitrate, when sprayed four to six times at 14 days interval, three weeks before harvest (Schumacher

,1978).Ford (1979) predicted that to increase fruit calcium by sprays. Calcium must directly penetrate the fruit; consequently, spray droplets must be deposited on the fruit to be effective in decreasing the occurrence of physiological disorder.

Fankhauser *et al.* (1979) found that bitter pit incidence in apple cultivars Cox's Orange Pippin and Gravenstein was reduced by air blast sprays of calcium chloride along with a fungicide. Spraying the orchard with Ca $(NO_3)_2$ upto six times greatly reduced the bitter pit from 50 % to below 5 %, and also reduced internal breakdown in apple (Delver, 1980). Fankhauser and Stadler (1981) reported that bitter pit of Gravenstein apples was controlled successfully with 20 kg $CaCl_2$/ha applied in 1000 litres of water; concentration higher than this damaged the leaves causing marginal scorching. Schumacher *et al.* (1981) found that three or six sprays of 1% calcium chloride and 0.4 % waxol suspension to cv. Marigold spindle trained apple trees were equally effective in controlling bitter pit. Healthy control and sprayed fruits both contained higher Ca level (27.5 mg/100g dry weight) and lower K:Ca ratio than fruit with bitter pit. The (K+Mg)/Ca ratio was significantly higher in flesh of fruit with bitter pit.

Ferguson and Watkins (1983) studied the distribution and concentration of Ca, Mg, and K in apples fruit sprayed with Ca during the growing season.Ca sprays increased Ca level in each tissue segment; K levels were lower in Ca sprays fruits, but effects on Mg were variable. Concentration of all three cations in the outer cortex increased during storage. Hartman (1983) also reported that spraying Cox's Orange Pippin Apple trees with 0.7 % $CaCl_2$ at 7 Kg/ha, 12 times from early July onward reduced the combined incidence of these disorders (Bitter pit and internal breakdown) to 2%, compared to 25% incidence in unsprayed controls.

Ben (1986) also observed that $CaCl_2$ treatment increased fruit calcium and improved flesh firmness decreased the incidence of Jonthan spot and core breakdown in Jonathan apples. Kallay *et al.* (1987) observed that water core development corresponded to a 23% decrease in Ca in fruits, 135 days after full bloom. Addition of $CaCl_2$ readily absorbed the sorbitol or glucose from the intercellular spaces of sorbitol or glucose treated fruits and transformed them to other monosaccharide's resulting in a reduction of water core (Filsowf, 1988). A field study conducted by Witney *et al.* (1991) indicated that $CaCl_2$ sprays slightly reduced bitter pit, increased fruit calcium reduced fruit magnesium and increased the number of apple per spur .The incidence of bitter pit ranged from 8% ($CaCl_2$ spray alone) to 88% (bagging +$MgCl_2$) sprays induced a wide range of bitter pit incidence, which could be reliably controlled by calcium sprays.

Desai (1980) reported significantly higher amount of nitrogen, potassium, magnesium and boron in the affected arils than in healthy arils in pomegranate fruit.

2.2. Inter fruit competition for water and nutrients and its effect on the fruit disorders

Stigter (1969), using a simplified plant system, clearly demonstrated the priority of fruit over vegetative growth in the cucumber. In tomato competition by the fruit may even lead to vegetative death when nitrogen is limiting (Murneek, 1926).

Competitive limitations on the growth rates begin as the increasing number of fruits mobilize nutrients supplies for their growth. The pattern of such mobilization is mainly determined by both the strength and proximity of the sink organs to the source (Szynkler, 1974; Walker and Ho, 1977; Cook and Evans, 1978). The inhibitory effect of growing fruits

on the development of pistils before anthesis were observed in cantaloupes (Rosa, 1924) and in okra (Perkins *et al.*,1952).

Hurd *et al.* (1979) reported that reducing sink activity by removing two third of the flowers in tomato resulted in larger plants with larger fruits. Similarly, Heuvelink and Buiskool (1995) observed that changes in dry matter distribution under high fruit load were correlated with lower leaf areas. These data suggest that leaves and fruits compete for assimilates.

Marcelis and Heuvelink (1990) showed that tomato fruit grown on plant with seven fruits per truss reached only 70% of the final dry weight of fruits grown on plants with one fruit per truss.

The source – sink alteration by fruit removal and shoot trimming had impact on yield, leaf sugar metabolism and grape composition (Mota *et al.*, 2010).

Inter fruit competition significantly limits the growth rates in chilli fruits as the increasing number of fruits mobilizes the nutrient available for growth. In experiment where competition was artificially modified by removal of buds , flowers and set fruits from first three nodes under open field and green house conditions, it was found that pericarp of fruits from non-competition treatment were much thicker than fruits from competition treatment (Ali and Kelly., 1992).

Kirti and Nettles (1961) reported that to produce high yield and good quality fruits, debudding the first five nodes of pepper plants was more effective than deflowering and defruiting.

Nyhlen and Rootsi (1961) reported that in case of apple fruit from trees with larger crops developing more pit than that from trees bearing smaller crops but in contrary, Whetzel (1912) and Smith (1926) reported

that the fruit on trees bearing light crops, which consequently produce more fruit, were more affected. The same trees had more bitter pit in productive years when the crop was light than in non-productive years when the crop was normal (Raphael and O'Loughlin, 1964).

Pawar *et al.* (1994) reported that pruning of tree canopy reduced aril browning incidence of pomegranate.

2.3. Plant growth regulators and their effect on development of fruit disorder

Plant hormones play a significant role in the processes that lead to mature fruit and viable mature seed (Nitsch 1970). Auxins, gibberellins (GAs), cytokinins, abscisic acid (ABA), and ethylene have been implicated at various stages of the fruit growth cycle (Crane 1964; Nitsch 1970; Pharis and King 1985; Gillaspy *et al.*, 1993).

Plant growth regulators help in making the developing fruit into a strong sink and mobilising nutrients from other parts of the plant into the developing fruits. Apart from the mobilisation of nutrients, these plant growth substances are also involved in cell division and cell enlargement during the growth and development of fruits (Salisbury and Ross, 1986).

Jahnke *et al.* (1989) monitored assimilate distribution in pea plants labelled with the short-lived isotope $^{11}CO_2$ on the axil leaf subtending pollinated or unpollinated fruit. The ^{11}C-radiolabel entering the ovaries paralleled the growth rate of the fruit. When the unpollinated ovaries stopped growing, the uptake of ^{11}C rapidly decreased. Pollination/ fertilization, however, restored the strong sink activity of the ovaries. The same effect could be achieved by applying GA_3 to unpollinated ovaries. About 2 h after treatment with GA_3, the ^{11}C-

radiolabel entering the ovary started to increase and, at about the same time, the ovary resumed growth.

Brenner and Cheikh (1995) stated that the concentration gradient of photoassimilates between source and sink tissue is likely the primary regulator for the current rate of transport and pattern of partitioning. Hormones may serve as modulators for many of the rate-limiting components in this process. Hormones may stimulate transport of nutrients through the phloem, modify the strength of the sink by stimulating its growth, and increase the ability for sucrose unloading from the phloem, or act on metabolism and compartmentalization of sucrose and its metabolites (Brenner and Cheikh, 1995).

In Seedless Clementine mandarin cultivars it has been shown that exogenous application of GA_3 increases fruit set. Further localized GA_3 treatment of pistil in citrus flower resulted in stronger mobilization of metabolites to young ovaries. Which appear essential for fruit set and development (Powell and Krezdorn, 1977) .

Growth regulatory activity of PBZ is reported to be associated with its potential to inhibit the biosynthesis of growth promotory substance, namely gibberellins (Graebe, 1987).

Curry (1988) noted that apple tree treated with PBZ on soil or foliar spray increased total soluble solids and reduced fruit acidity. In peach PBZ was more effective in increasing fruit yield/tree. Paclobutrazol may decrease fruit cell division during the first stage of fruit growth and improve fruit cell enlargement during the following stage.

The occurrence of water core in the Japanese pear cultivar 'Hosui' increased the GA_3 activity in fruit. Palcobutrazol or Prohexadione Ca, a

GA_3 synthesis inhibitor, decreased the occurrence of water–core in 'Hosui' pear (Yuji *et al.*, 1996).

2.4. Significance of seed in fruit disorder

Seeds are well known to be a rich source of plant growth regulators (Hedden and Hoad, 1985). As a result of auxin production seeds of a fruit may affect competition between fruits, either by increasing the sink strength (competitive ability to attract assimilates) of the fruit, or by suppressing the sink strength of other fruits (Varga and Bruinsma, 1976). In the first situation an increase in seed number of the first fruit may reduce growth of the second fruit because of competition for limited assimilate supply, while in the second situation growth reduction is due to hormones produced by older fruits (dominance).

Viable seed are an important sink signal for driving fruit development and seed fill and is often correlates with yield (Egli, 1994).

In many species the weight of the fruit tissue increase with an increasing number of well developed seeds and in some cases it has been possible to kill or remove the seeds and replace them with growth substances which restored normal fruit development i.e. Peas (Eeuwens and Schwabe, 1975) and *Rosa arvensis* (Jackson,1968).

Ravindra and Shivashankar (2004) conducted studies on the significance of *in situ* seed germination events and its effect on spongy tissue development in mango and found that there was a considerable difference in the number of days taken for germination between affected and healthy fruit. According to their observation, the affected fruit seed took less number of days for germination than the healthy fruit seed. They observed that small percentage of the fruit also exhibited vivipary.

The first report on the causative role of seed in the formation of spongy tissue was made by Ravindra and Shivashankar (2004). According to them, spongy tissue in Alphonso mango is triggered by the onset of seed germination associated events. These events lead to development of spongy tissue in the pulp close to the stone by the continuous transfer of water from pulp to the germinating seed.

Ravindra and Shivashankar (2004) observed a significant difference in various biochemical parameters between spongy tissues affected fruit seed and healthy fruit seed. According to them, Moisture content was significantly higher in seeds from spongy tissue affected fruits than that from healthy fruits, while there was a corresponding decrease in the spongy tissue. Analysis of other components revealed that seeds from spong-tissue-affected fruits had significantly lower starch content (33.5%) and higher levels of soluble sugars (27.7%) than healthy fruits. A significant rise in the content of both DNA (76.7%) and RNA (65.5%) in spongy tissue affected fruit seed indicated the rapid progress in germination. A substantial increase in the content of soluble protein (43.1%) in spongy tissue seeds indicated *de novo* synthesis of various enzymes associated with germination. The phenols also increased substantially (51.6%) due to high metabolic activity. There was a significant increase in spongy tissue incidence in pre-harvest treatment of fruits with GA_3 (70.2%), while there was considerable reduction in incidence with paclobutrazol (16.4%) compared to 51.5% incidence in the control. GA_3 treatment also resulted in higher intensity of spongy tissue, where > 70% of the fruit mesocarp was affected. The amylase and lipase activity in affected fruit seed was significantly higher than the healthy fruit seed. From these studies it was concluded that spongy tissue in Alphanso mango was triggered by the initiation of germination events (Shivashankar *et al.*, 2007).

2.5. Biochemical changes in fruit in relation to development of physiological disorder

Biochemical studies of spongy tissue affected pulp in mango is done by Gupta *et al,* (1985) indicate that the affected pulp had higher acid to sugar ratio, higher starch, polyphenol and reduction in non reducing sugar, caretenoids, ascorbic acid and protein.

Selvaraj *et al.* (2000) observed that the spongy tissue had low carotenoid, total sugar and sugar: acid ratio, Ca, K, and higher dry matter, alcohol insoluble solids, starch, acidity and glucose: fructose ratio compared to healthy tissue. He found that breakdown tissue had twice the citric acid and half the amount of malic acid as compared to healthy tissue of mango.

Shivashankar *et al.* (2004) suggested that the browning of arils in pomegranate resulted in lower starch and acid metabolism.

Tobar *et al.* (2009) monitored disordered (aril browned) pomegranate fruit and healthy fruits, in the 131st days of fruit set. Disordered fruits showed a significant ($P < 0.05$) difference from intact fruits in fruit density, juice percentage, phenolic compounds, polymeric anthocyanins, titratable acidity, and total soluble solids in the 131st days of fruit set. The fruit density, juice percent, ascorbic acid, phenolic compounds, and titratable acidity decreased, faster than those in intact fruits. Peel percent, dry matter of juice, acidity, total soluble solids and total sugars increased faster than those in intact fruits, The highest protein contents were 219 mg/100 g in intact fruits at the 159th day of fruit set and 211 mg/100 g at the 152th day of fruit set in disordered fruits. Results indicated that changes in physical and chemical properties in disordered fruits were faster than those in intact fruits.

In pears, a relationship was found between ascorbic acid content and the susceptibility to browning during experimental storage under various brown core-inducing condition (Veltman *et al.*,1999).'Conference' pears tend to develop tissue disorders, like brown core, when ascorbic acid levels drop below a certain value. Decline in ascorbate concentrations is often associated with reduced capability to prevent oxidative damage (Noctor and Foyer, 1998).

Sawant (1993) found that the spraying of ascorbic acid on the pomegranate plants as an antioxidant showed a significant effect on all biochemical parameters of blackening suggesting that treatment of pomegranate fruits with an effective antioxidant like ascorbic acid may reduce aril browning malady.

Desai (1989) reported that the affected arils were low in TSS, ascorbic acid, acidity, total and reducing sugars and carbohydrates but high in non-reducing sugar and starch as compared to healthy arils. The tannins content of affected arils was also high and the increase ranged from 92.6 to 385%. Further, Sawant (1993) also observed reduction in TSS acidity, reducing and total sugars and free amino acids. Shete (1998) observed in affected arils that there was reduction in TSS, reducing and total sugars and ascorbic acid content of non-reducing sugar, tannin and boron as compared to healthy arils of both healthy and affected fruits.

Post harvest browning of litchi was thought to be caused by a rapid degradation of the red pigment by polyphenol oxidase (PPO), producing brown coloured by-product (Akamine, 1960). Recently Jiang (2000) reported that litchi PPO cannot oxidize anthocyanin, but the anthocyanin might be degraded rapidly in an anthocyanin –PPO- phenol system and, thus, suggested that it may be the presence of the sugar moiety which caused steric hindrance. Since anthocyanins are unstable; they could be

degraded nonenzymatically or enzymatically. In addition, anthocyanase (anthocyanin-β-glucosidase) could play a role in removing the sugar groups, leading to the anthocyanin decolourization (Huang, 1990).

It has been reported that TSS and total sugars increased significantly during the maturation stage in fruits such as pomegranate, mango, and banana (Bashir and Abu-Goukh, 2003; Kulkarni and Aradhya, 2005). It may be attributed to hydrolysis of starch into simple sugars (Biale, 1960). Similar trend has been reported by Tobar *et al* in pomegranate in intact fruits, but it seems that an unknown factor increased the accession of TSS and total sugars in disordered fruits (aril browned fruits). The sugar moiety of anthocyanins is thought to be the steric hindrance against PPO attack, and thus, there was a low affinity for PPO (Zhang *et al.*, 2001). Zhang *et al.* (2005) indicated that the anthocyanase could first remove the sugar moiety from litchi anthocyanins, producing anthocyanidins, and finally POD causes the degradation of the anthocyanidin in the presence of H_2O_2. Sugars and their degradation products accelerate the degradation of anthocyanins. Fructose, arabinose, lactose, and sorbose have greater degradative effects on anthocyanins than glucose, sucrose or maltose (Hendry and Houghton, 1996).

Zauberman *et al.* (1991) reported that polyphenol oxidase may play an important role in degradation of the pigment molecules of anthocyanin while peroxidase did not have this role. Many factor involved in chemical decoloration of anthocyanins including pH, temperature, ascorbic acid, sugar, oxygen and metallic ions (Eskin, 1979). Under acidic condition pH 2.0 and below, the colour of anthocyanin solution increases, the colour tends to change, usually to blue or purple, although sometimes yellow colours are obtained.

Polyphenol oxidase and peroxidase enzymes were reported to be involved in enzymatic browning of pears, litchi and apple (Underhill *et al.*,1992; Richard and Gauillard, 1997).Increased oxidative damage and higher activities of antioxidative enzymes were also reported to be the major reason for browning in pear (Larrigaudiere *et al.*, 2001). A significant increase in the activities of superoxide dismutase, polyphenol oxidase and peroxidise was observed in brown arils compared to healthy arils by Shivashankar *et al.* (2004). Similar increase in the activities of polyphenol oxidase, superoxide dismutase enzymes and malondialdehyde content have been reported in browned peels of pomegranate (Xinghua *et al.*,1998).

The activities of polyphenol oxidase and peroxadise were found to increase in affected arils while, the activity of catalase and α–amylase was significantly reduced by Prabhu- Desai (1989) and Sawant (1993).

Franck *et al.* (2007) suggested that browning disorder of pear was caused by an imbalance between oxidative and reductive processes due to metabolic gas gradient inside the fruit. This may lead to an accumulation of reactive oxygen species which in turn, may induce loss of membrane integrity which becomes macroscopically visible through the enzymatic oxidation of phenolic compound to brown coloured polymers.

Shivashankar *et al.* (2004) suggested that the browning of arils in pomegranate can be attributed to the higher oxidative damage of membrane leading to higher activities of certain enzymes like polyphenol oxidase and peroxidase.

Material and Methods

III. MATERIALS AND METHODS

3.1. Fruit samples

Pomegranate fruit of cv. Bhagwa, at different developmental stages, namely, 84-, 98-, 112- and 126-day old from the day of fruit set, were harvested from a pomegranate orchard located in Sira, Tumkur district, Karnataka, for all the studies collected during the season of 2010-2011.

3.2. Tagging

Fruits were tagged at 40% maturity (56 days from fruit set) stage in orchard located in the Sira district, Karnataka, according to different location and treatment of fruit.

3.3. Treatments

- Pre-harvest treatment of immature fruit (at 40%, 50%, 60%, 70% and 80% maturity) with GA_3 (1000ppm), PBZ (2000ppm) and $CaCl_2$ (5%) were carried out, 5 replications taken for each of the treatment.

3.4. Chemicals

Dinitrosalicylic acid reagent (DNS) and Tetrazolium salt were obtained from SD Fine Chemicals Company, Bangalore; Bovin serum albumin was obtained from Qualigen Fine Chemicals, Mumbai. All the chemicals obtained were of the AR grade available commercially.

3.5. Sample collection

All treated samples were collected from the selected pomegranate plant in the orchard at different maturity of fruit during the season. Various physiological parameters in the pulp and seed of the fruits were analyzed using standard procedures.

Fig. 2. Tagging of fruits in orchard

3.6. Estimation of total moisture content

The moisture content of the pulp and seed samples was analyzed by the gravimetric procedure. Known quantity of the pulp and seed were dried at 70^{o}C in a hot air oven. The weight of the sample after drying was recorded and the moisture was expressed on percentage basis.

3.7. Biochemical analysis

For all biochemical analysis 5 replications were taken.

3.7.1. Estimation of total soluble sugar

Reagents

1. Dinitrosalicylic acid (DNS) Reagent :- Dissolve by stirring 1g DNS, 200 mg crystalline phenol, 50 mg sodium phosphate and 20 g Rochelle salt (sodium potassium tartarate) in 100 ml 1% NaOH.
2. 80% ethanol
3. Concentrated HCl
4. Phenolphthalein indicator
5. Standard glucose solution:-100mg of glucose was dissolved in 100 ml of water. 1ml of this solution was diluted to 10 ml with water to get the standard containing 100µg/ml.

Sample extraction

1g of the fresh sample was extracted in 10ml of warm 80% ethanol. The extract was centrifuged at 10,000rpm for 10 min. The supernatant was evaporated on water bath to dryness and the residue was dissolved in 10ml of water. This alcohol-free extract was used for estimation of total soluble sugars (AOAC, 1965).

Estimation

To 10ml of the residue with water, 1ml of concentrated HCl was added and left over night for complete hydrolysis. To this reaction mixture 2-3 drops of 0.5% phenolphthalein indicator was added and neutralized with 40% NaOH. The volume of the neutralized solution was made up to 25 ml. Aliquots of 0.2 ml and 1 ml were taken for estimation of sugars by using DNS reagent 0.2 ml of the aliquot was diluted to 1 ml with water and to this 0.5 ml of DNS reagent was added and heated for 5 min. The reaction mixture was cooled under running tap water and volume was made up to 20ml. The absorbance of the solution was recorded at 540 nm against the reagent blank.

A standard graph was constructed with glucose as standard in the range of 20 to 100 µg. The results were expressed mg glucose/g of sample.

3.7.2 Estimation of reducing sugar content

Reagents

1. Dinitrosalicylic acid reagent (DNS): Dissolve by stirring 1g DNS, 200 mg crystalline phenol, 50 mg sodium phosphate and 20 g Rochelle salt (sodium potassium tartarate) in 100 ml 1% Na0H.
2. 80% ethanol
3. Standard glucose solution 100mg of glucose was dissolved in 100 ml of water. 1ml of this solution was diluted to 10 ml with water to get the standard containing 100µg/ml.

Sample extraction

1g of the fresh sample was extracted in 10ml of warm 80% ethanol. The extract was centrifuge at 10,000 rpm for 10 min. The supernatant

was evaporated on water bath to dryness and residue was dissolved in 10ml of water.

Estimation

An aliquot of 0.5 ml was diluted with water to 1.0 ml and to this 0.5 ml of DNS reagent was added and heated for 5 min. The reaction mixture was cooled under running tap water and volume was made up to 20 ml. The absorbance of the solution was recorded at 540 nm against the reagent blank.

A standard graph was constructed with glucose as standard in the range of 20 to 100 µg. The results were expressed as glucose/g (AOAC, 1965).

3.7.3. Estimation of starch content

Reagents

1. 80% ethanol
2. 52% perchloric acid
3. Standard glucose solution: 100mg of glucose was dissolved in 100 ml of water. 1ml of this solution was diluted to 10 ml with water to get the standard containing 100µg/ml.
4. Dinitrosalicylic acid reagent (DNS): Dissolve by stirring 1g DNS, 200 mg crystalline phenol, 50 mg sodium phosphate and 20 g Rochelle salt (sodium potassium tartarate) in 100 ml of 1 % NaOH.

Sample extraction

1g of the fresh sample was extracted in 10ml of warm 80% ethanol. The extract was centrifuge at 10,000 rpm for 10 min. The residue was retained and air dried well. The dried residue, 5 ml of water and 6.5 ml of 52% perchloric acid were added and stirred for 20 min at 4^0C, the extract

was centrifuged at 5,000 rpm for 15 min and the supernatant was used for the estimation of starch (AOAC, 1965).

Estimation

An aliquot of 0.5 ml was diluted with water to 1.0 ml and to this 0.5 ml of DNS reagent was added and heated for 5 min in boiling water bath. The reaction mixture was cooled under running tap water and volume was made up to 20 ml.

The absorbance of the solution was recorded at 540 nm against the reagent blank.

3.7.4 Amylase activity

Amylase activity was determined by DNS method. The product of amylase activity was allowed to react with DNS reagent, which formed an orange- red colour product when starch was used as the substrate. Maltose, the reducing sugar, which was formed by the action of amylase, was determined colorimetrically at 540 nm (Bernfeld, 1955).

Reagents

1. Acetate buffer, 0.016M, pH 4.7.
2. Starch 1% solution: A fresh solution was prepared by dissolving 1 gm starch in 100 ml acetate buffer with slight warming when required.
3. DNS reagent: Dissolve by stirring 1g DNS, 200 mg crystalline phenol, 50 mg sodium phosphate and 20 g Rochelle salt (sodium potassium tartarate) in 100 ml of 1% NaOH.
4. Maltose solution: Dissolve 50 mg maltose in 50ml distilled water in standard flask and stored in refrigerator.

Extraction of amylase

Fresh sample (1g) was extracted with 0.016 M acetate buffer (4.7). The extract was centrifuge at 10,000 rpm for 15 min. The supernatant was used as source of amylase.

Amylase assay

The diluted enzyme extract (0.5ml) was incubated for 30 min at 20^{o}C with 0.5 ml of substrate solution. The enzyme reaction was terminated by the addition of 0.5ml DNS reagent. The tube containing this mixture was heated for 5 min in boiling water bath and then cooled in running tap water. An experimental blank was prepared similarly except that the DNS reagent was added to the reagent mixture immediately after the radiation of substrate after incubation. A standard graph was constructed with maltose as a standard in the range of 0.2 to 2mg.

Expression of amylase activity

Amylase activity in the sample was expressed as mg maltose liberated/h/g of protein.

3.7.5. Total dehydrogenase activity

Reagents

- 2, 3, 5,- triphenyltetrazolium chloride (TTC)
- 70 % methanol

Sample extraction

0.03g of sample was ground with 1.5 ml of 70 % methanol with the help of mortar and pestle. The extract was centrifuged at 10,000 rpm for 10 min in room temperature (Sung and Chen, 1988).

Total dehydrogenase assay

The seed coat was removed and soaked in distilled water and incubated at over night. Following next day endosperm was incubated with 3ml of 1% TTC solution (0.1% for the embryo). The tubes were incubated for 18 hrs at 37 ^{o}C in a water bath. After incubation the samples were taken and ground with 1.5 ml of 70% methanol. Centrifuge rated at 10,000rpm for 10 min at room temperature. Absorbance was recorded in a spectrophotometer at 485 nm. Dehydrogenase activity was expressed as OD units per 100mg of tissue.

3.7.6 Estimation of total soluble protein

Reagents

Reagent A: 2% Na_2CO_3 in 0.1 N NaOH .

Reagent B: 0.5 % $CuSO_4$. $5H_2O$ in 1% sodium potassium tartarate.

Reagent C: Mix 50 ml of reagent A with 1 ml of reagent B. Make fresh each time.

Reagent D: 1 part of Folin- Ciocalteau phenol Reagent+ 2 part of water

Standard bovine serum albumin (BSA) solution BSA (2gm) was dissolved in water to get a working standard solution containing 200 µg / ml

Procedure

0.5 ml sample (10- 200 µg) protein was combined with 5.0 ml reagent C in a culture tube, mixed well, and left at room temperature for 10 min. reagent D was mixed. Absorbance was measured in the spectrophotometer at 660nm (Lowry *et. al.*, 1951).

3.6.7. Estimation of Anthocyanin

Reagent

1. HCl : Methanol:- 1:99 (Acidic methanol)

Procedure

2.5g of sample was taken and homogenised with Acidic Methanol (AM) and kept for 72hrs. The mixture was squeezed and the residue was re-extracted 2 to 3 times to extract maximum quantity of anthocyanin. The extract was pooled and made up the volume with AM to 50ml. Absorbance was measured in the spectrophotometer at 540nm adjusting 100% transmission against AM. Amount of anthocyanin in unknown sample can be expressed as mg/100g fresh weight using standard cyanidin hydrochloride as standard.

Calculation

$$\text{Anthocyanin(mg/100g)} = \frac{OD_{540} \times \text{Standard OD} \times \text{volume made}}{\text{Weight of sample (g)}} \times 100$$

3.6.8. Estimation of Total phenol

Total phenol content is estimated by spectrophotometric method using Folin Ciocalteu Reagent (FCR) method. Phenol react with an oxidizing agent phosphomolybdate in Folin – Ciocalteau Reagent under condition and result in the formation of a blue coloured complex , the molybdenum blue which was measured at 700nm.

Reagent

1. 80% methanol
2. Folin and Ciocalteau's Phenol Reagent (1N)
3. 20% Sodium Carbonate (Na_2CO_3)
4. Standard Phenol (Gallic acid solution)

Procedure

5g of sample was extracted using 80% methanol with the help of pestle and mortar repeatedly three times. The extract was pooled and made up the volume to 50 ml. 0.5ml of freshly prepared sample was taken in the test tubes. 0.2 ml of FCR reagent added to all the tubes. 3.3ml of distilled water was added to all the tubes. All the tubes are mixed well. Then, 1ml of Sodium Carbonate solution was added to all the test tubes .Then, the tubes were kept in incubation at room temperature for 30 minutes. The colour so developed was read spectrophotometrically at 700nm. Standard curve of phenols using Gallic acid (GA) as standard (Malick and Singh, 1980)

Calculation

$$\text{Total phenol (mg/100g of aril)} = \frac{OD_{700} \times \text{Standard value (mg/OD)} \times \text{volume made}}{0.5 \times \text{weight of sample (g)}} \times 100$$

3.6.9. Estimation of Total Titrable Acidity

Reagent

1. Standard NaOH solution (N/100).

2. Phenolphthalein indicator (0.5% in alcohol).

Procedure

2g of sample was homogenised with water in pestle and mortar and squeezed through layer of muslin cloth. Same process was repeated twice and all the extract pooled together and mixed well. Final volume was made up to 50ml. The filtrate was titrated against standard N/100 NaOH solution using phenolphthalein as indicator (AOAC,1970).

Calculation

$$100 \text{ gm contains acidity} = X \times \frac{100 \times 100 \quad \text{ml of N/100 NaOH}}{10 \times 10}$$

Or = X mill equivalent per 100gm sample

1 mill equivalent = 1 ml of 1N solution

1ml of NaOH = 68.75 mg of Citric acid

= 68.83 mg of Malic acid

= 73.26 mg of Tartaric acid

3.7.10. Estimation of Polyphenol Oxidase

Reagents

1. 0.05 M Citrate Phosphate buffer, pH 6.8.
2. 1.25 % Pyrogallol.

Procedure

0.3g of seed sample was grinded with ice cold acetone and filtered with muslin cloth and residue was dried on Whatman's No.1 filter paper. Then dry weight of acetone powder was taken. Then known weight of acetone powder was taken and 1ml of 0.05M Citrate phosphate buffer of pH 6.8 was added and kept for overnight. Next day it was homogenized with same buffer and then centrifuged at 10,000rpm for 10 minutes (Esterbaner, 1977).

Assay

For blank

0.05M citrate phosphate buffer (pH 6.8) - 3.5ml

For substrate blank

0.05M citrate phosphate buffer (pH 6.8) - 3.4 ml

1.25 % pyragallol - 0.1ml

For enzyme

0.05M citrate phosphate buffer (pH 6.8)	-	2.9ml
1.25 % pyragallol	-	0.1ml
Enzyme extract	-	0.5 ml

Enzyme blank

0.05M citrate phosphate buffer (pH6.8)	-	3.0 ml
Enzyme extract	-	0.5 ml

The reaction was studied with pyragallol. OD change was measured at 412nm/min or per second. Polyphenol oxidase catalyses the oxidation of monophenols and p-diphenols.

3.7.11. Estimation of Ascorbic acid by 2,6 - dichlorophenol indophenols Method

Vitamin C can be estimated using DCPIP dye which is blue in colour but becomes colour less when reacts with ascorbic acid. Therefore, the end point for the estimation of ascorbic acid will be the appearance of pink colour. Ascorbic acid is more stable in acidic medium. Therefore, extraction is usually done in mild acidic medium like metaphosphoric: acetic acid or oxalic acid.

Estimation by titrimetric method using oxalic acid

Titrimetric estimation of vitamin C is conventionally done using 2, 6-dichlorophenol Indophenol dye solution. This dye is blue in alkaline solution and pink in acidic solution. Ascorbic acid reduces the dye to colourless form. Reaction is quantitative and specific for ascorbic acid at pH 1.0 – 3.0. The end point is the appearance of pink colour (Sadasivam and Theymoli,1987).

Reagent

1. 4% oxalic acid.
2. 2,6-dichlorophenol Indophenol dye solution : 0.025 per cent (50mg) 2, 6-dichlorophenol Indophenol was weighed and taken in a beaker. 4.2 mg Sodium Bicarbonate was added to it. It was then dissolved in little amount of distilled water and the volume was made up to 200ml with distilled water.
3. Standard Ascorbic acid solution: 0.01% in 4% oxalic acid.

Procedure

1. 5g of sample was extracted in 4% oxalic acid and made up to 50ml and centrifuged.
2. 5ml of extract solution was taken and made up to 15 ml by the addition of 4% oxalic acid.
3. A burette was filled with indophenol dye solution and titrated against the sample extract (V_2).
4. End point was determined by the appearance of pink color which should persist for about 15 seconds.
5. Standardization of dye solution: 5ml of standard ascorbic acid solution was taken in a conical flask and to this 5ml of 4% oxalic acid solution was added. The contents of the conical flask were thoroughly mixed by shaking and titrated (V_1) against indophenol dye solution to get the pink colour end point.

Calculation

$$\text{Amount of Ascorbic acid (mg/100g)} = \frac{\text{Amount of A. acid present in 5ml (in mg)} \times V_2 \text{ ml} \times 100}{V_1 \text{ ml} \times 5 \text{ ml} \times \text{wt of sample (g)}} \times 100$$

3.8. Incidence of aril browning

- **Percent incidence (%)**: Percent incidence was calculated as ratio of weight of browned aril to the total weight of aril multiplied by 100.

3.9. Intensity of aril browning

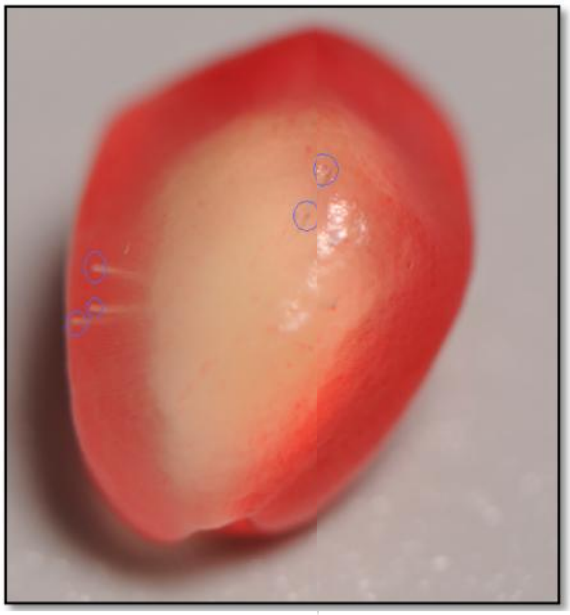
- **Low Intensity (LI)**: represents occurrence of small whitish to greyish dots of the size of a pin head on the aril.

- **Big spot (BS) or Medium Intensity (MI)**: represents browned spots on the aril with a diameter ranging from 1 -2 mm.

- **High Intensity (HI):** represents incidence of aril browning where more than 50 % area of the aril was affected by browning some of which were shrivelled also.

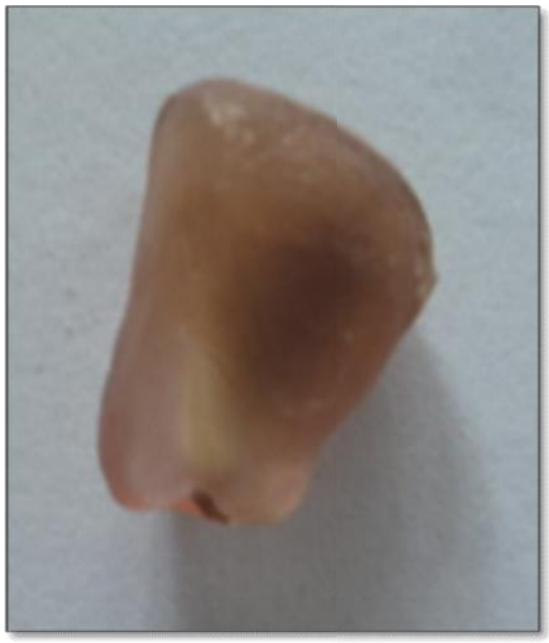
3.10. Statistical analysis

The data was statistically analysed by subjecting to ANOVA as described by Sundararaj *et al.* (1972). Critical difference values were compared at 1% and 5% levels of significance and whenever 'F' was found significant, treatment means were compared.



(A)Aril showing Low intensity (LI) of aril Browning (AB)

(B)Aril showing Medium Intensity or Big Spot (BS) of AB



(C)Aril showing High Intensity (HI) of aril browning

Fig. 3. Arils showing different intensity of browning

Experimental Results

IV. EXPERIMENTAL RESULTS

This chapter describes the results of the effect of fruit location, fruit maturity, fruit number per panicle, harvest date on the development of disorder, biochemical changes occurring during aril browning and effect of growth hormone treatment on development of disorder.

4.1. Effect of fruit location on the incidence of Aril Browning (AB)

The effect of fruit location on the incidence of aril browning (AB) is presented in Table 3a. The result showed that the aril browning incidence was more in case of fruits which were located inside the canopy (15-BS, 3-HI per 100 aril examined) as compared to fruits exposed to sun (BS-5, HI-1 per 100 aril examined). Table 3b. represents differences in incidence of aril browning due to fruit location on main shoot and lateral shoot. The result showed that incidence of aril browning was more in case of fruits located on lateral shoots (BS-16, HI-2 per 100 aril examined) as compared to fruits located on main shoot (BS-8, HI-1 per 100 aril examined).

4.2. Influence of fruit load on the incidence of aril browning

The effect of number of fruit per panicle and its effect on aril browning incidence are presented in Table 4. The result showed that the aril browning incidence was more in case of two fruits per panicle (13-BS, 2-HI per 100 aril examined) as compared to one fruit per panicle (7-BS, 1-HI per 100 aril examined).

4.3. Effect of maturity on the incidence of aril browning

The relationship between percent incidences of aril browning with the increasing in maturity of the fruit is presented in Fig.4. Results revealed that the fruits harvested at higher maturity (90%) were highly

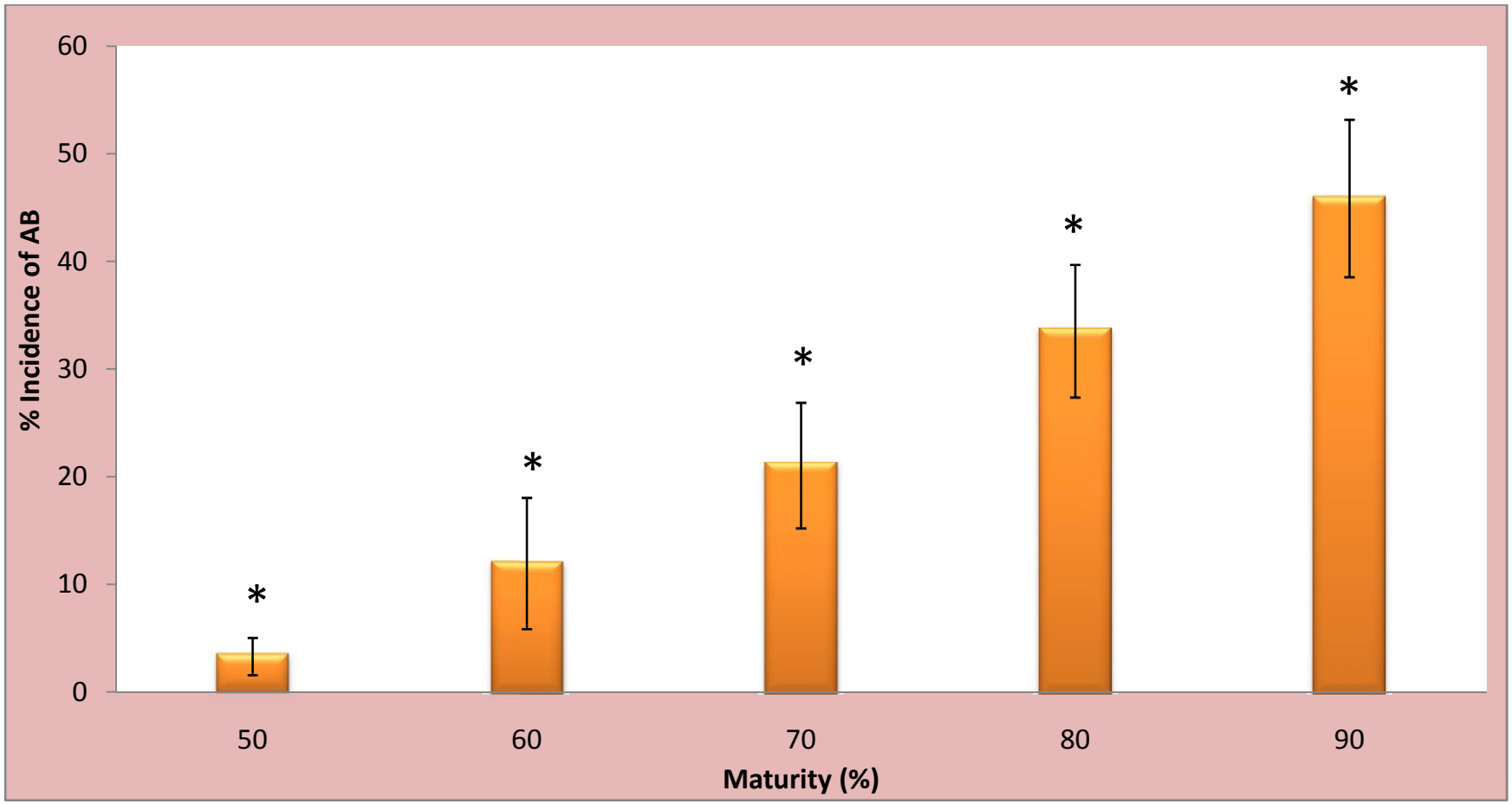


Fig. 4. Relationship between harvest date (maturity) and incidence % of AB

(I) **Vertical bars represent Standard Error (SE)**

Significant at1% (CD 1% = 10.35, SEm(±) 2.573)

affected by aril browning (45.85 %) than those harvested at 50% maturity (3.3%).

4.4. Difference in Germinability between healthy and aril browning affected seed as a function of time

The data on relationship of germination percent in healthy and aril browning affected seeds in different days after sowing is presented in Fig.5.

The results showed that the seed from healthy arils took less time in germination than the seed of aril browning affected seed. Result also showed that in the seed from healthy aril germination percentage was higher (92 %) than the seed from aril browning affected aril (74 %).

Table 3a. Effect of fruit location on the incidence of aril browning

Tissue status	No. of arils affected with browning per 100 arils examined	
	Fruits exposed to sun (No. of arils affected by AB)	Fruits inside canopy (No. of aril affected by AB)
Big spot (BS)	5	15
High Intensity (HI)	1	3
F-test	**	
CD value at 91%	3.74	
SEm(±)	0.86	

** Significant at 1%

Table 3b. Effect of fruit location on the incidence of aril browning

Tissue status	No. of arils affected with browning per 100 arils examined	
	Fruit on lateral shoot (No. of arils affected by AB)	Fruit on main shoot (No. of arils affected by AB)
Big spot (BS)	16	8
High Intensity (HI)	2	1
F-test	**	
CD value at 1%	4.10	
SEm(±)	0.94	

Table 4. Effect of fruit load on the incidence of aril browning

Tissue status	No. of arils affected with browning per 100 arils examined	
	One fruit/panicle (No. of arils affected by AB)	Two fruits/ panicle (No. of arils affected by AB)
Big spot (BS)	7	13
High Intensity (HI)	1	2
F-test	**	
CD value at 1%	4.24	
SEm(±)	0.98	

****** Significant at 1%

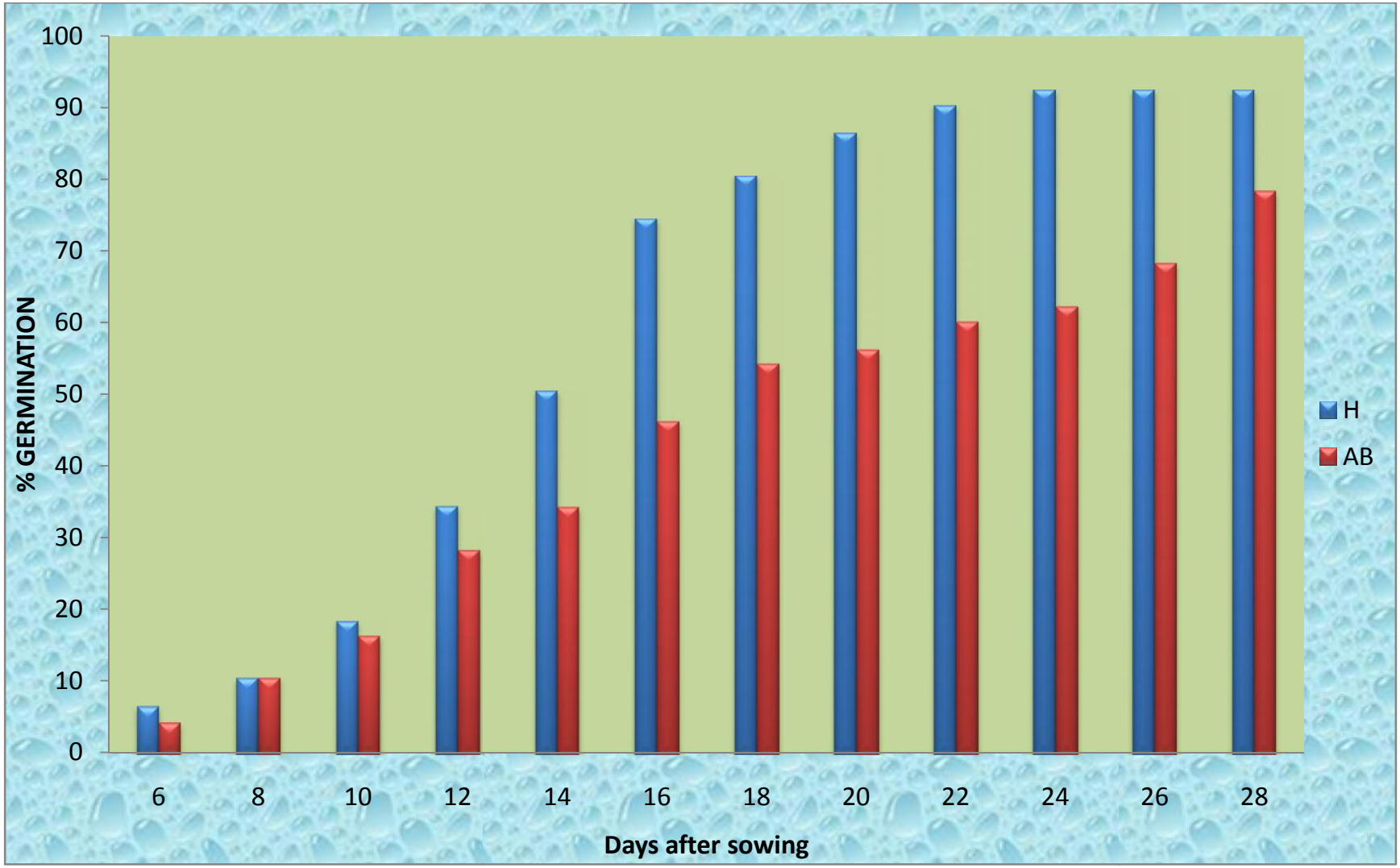


Fig. 5. Germinability of seeds collected from healthy and AB affected aril

4.5. Physiological changes

4.5.1. Moisture changes in healthy aril and AB affected arils

Moisture changes in healthy aril and AB affected aril are presented in Fig.6. and Fig.7. The results showed that the moisture content in pulp of AB affected aril was lower (82.33%) compared to the moisture content in healthy pulp of the same fruit(83.56%). Seed also showed a considerable difference in moisture content of seed of healthy aril(48.11 %) and seed of affected aril (46.22%).

4.6.1. Carbohydrate changes in healthy and aril browning affected aril juice and seed

4.6.1.1. Total soluble sugars in healthy and aril browning affected aril juice and seed

Table 5 and Table 6 represent total soluble sugars in healthy and aril browning affected aril juice and seed of pomegranate. The results showed that the total sugar content in juice of aril browning affected aril was higher than that of juice from healthy aril of the same fruit. Seed also showed a considerable difference in total sugar content in seed of healthy and AB affected aril.

Changes in total soluble sugar with increasing intensity of aril browning are presented in Fig.8. and Fig.9. The result showed that there was an increase in total soluble sugar with increase in intensity of aril browning. The lowest total soluble sugar for healthy seed and juice from healthy aril were recorded as 11.10mg/g of tissue FW and 67.35mg/g of tissue FW respectively, which increases to 28.56 mg/g of tissue FW and 84.21 mg/g of tissue FW for seed and juice from high intensity of aril browning affected aril respectively.

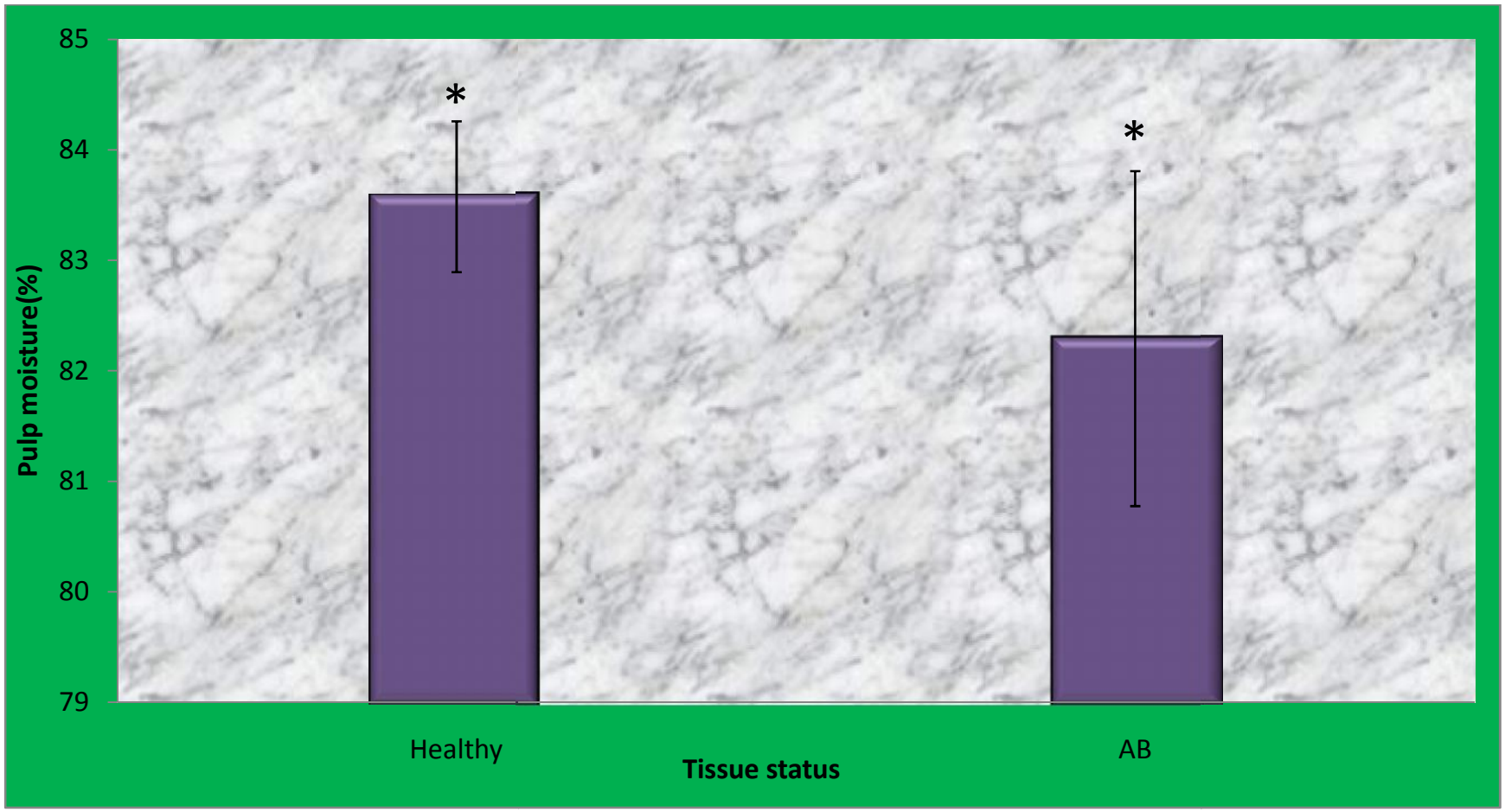


Fig. 6. Moisture content in pulp of healthy aril and AB affected aril

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 1.829, SEm(±) 0.3856)

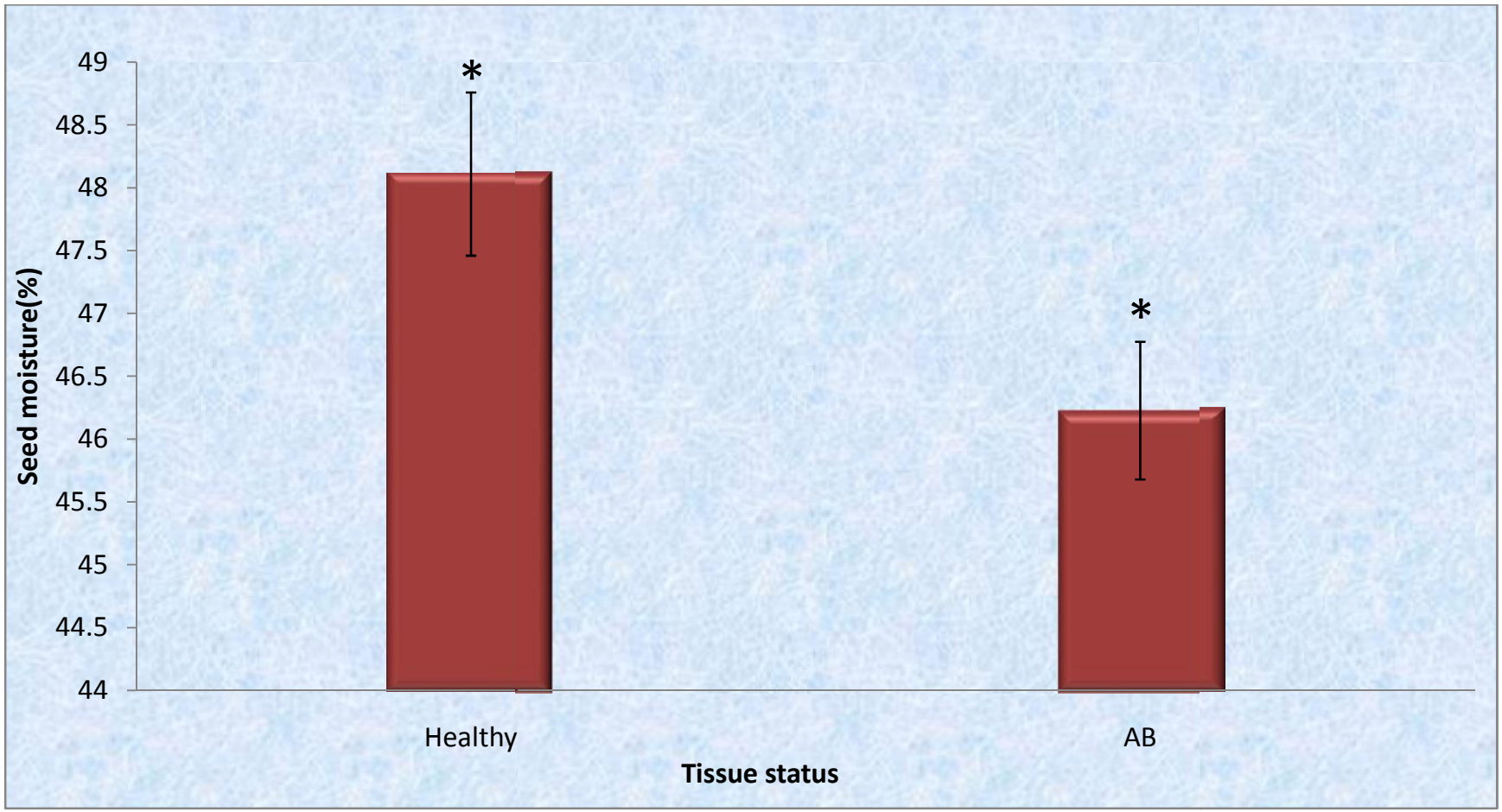


Fig. 7. Moisture content in seed of healthy aril and AB affected aril

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 0.7367, SEm(±) 0.1553)

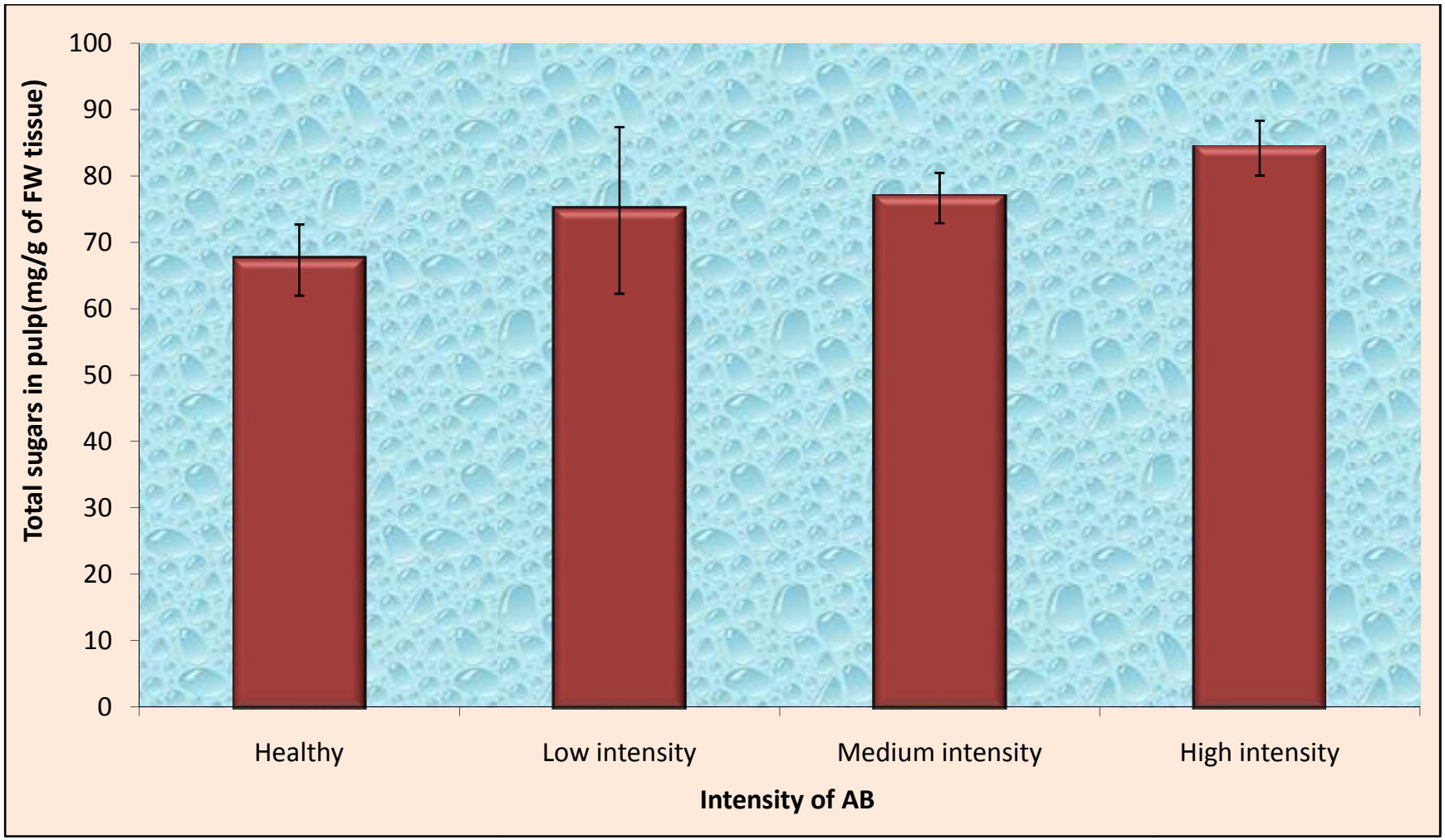


Fig. 8. Changes in total sugar in pulp with increasing intensity of AB

(I) **Vertical bars represent SE**

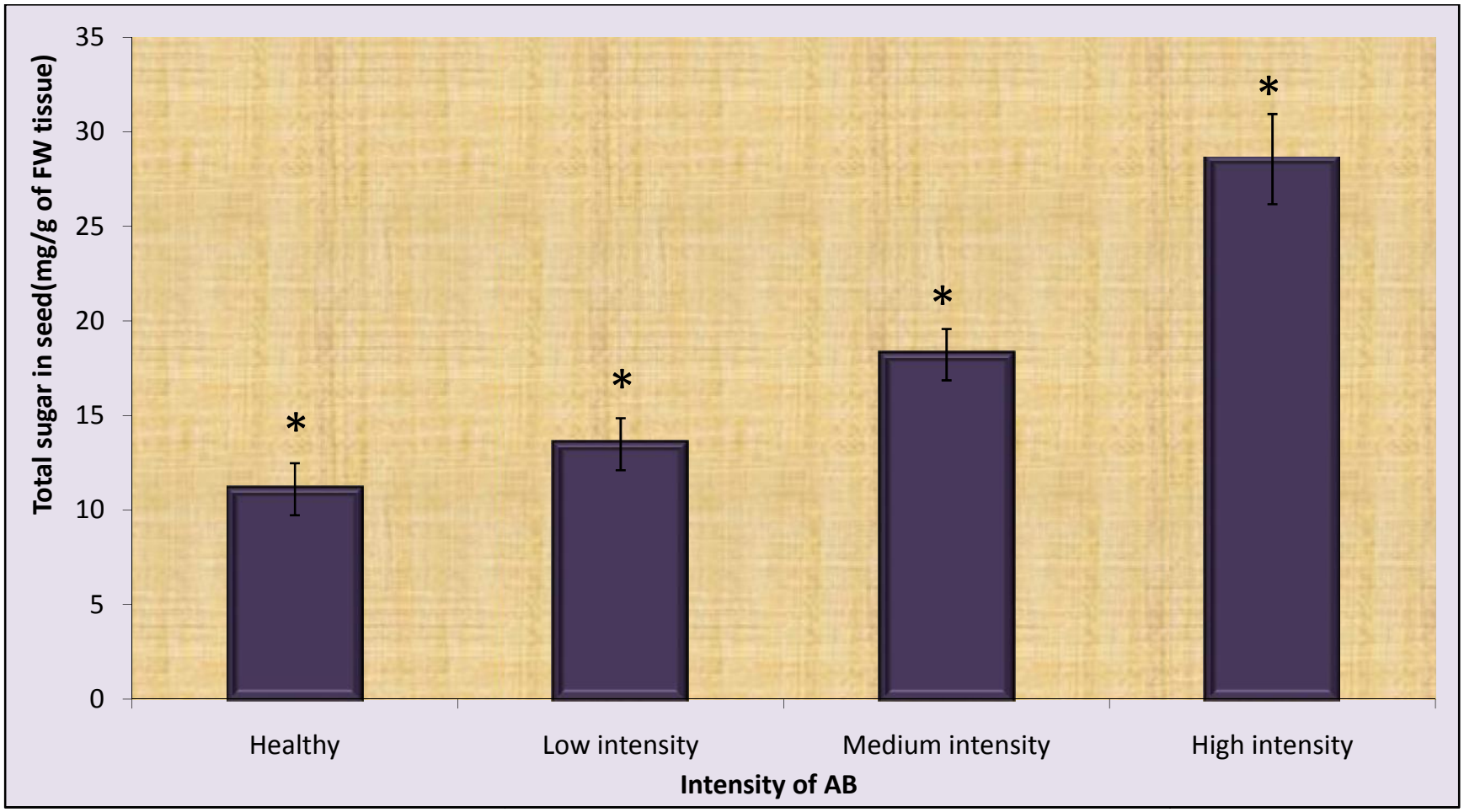


Fig. 9. Changes in total sugar in seed with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 4.620, SEm(±) 0.9739)

4.6.1.2. Reducing sugar content in healthy and aril browning affected aril juice and seed

Table 7 and Table 8 represents reducing sugar in healthy and AB affected aril. Results showed that the there was significant variation among healthy and aril browning affected aril juice and seed. Aril browning affected aril juice and seed recorded higher reducing sugar content (reducing sugar of juice of browned aril and seed was 53.40 mg/g of tissue FW and 21.75 mg/g of tissue FW respectively) than the healthy aril juice and seed (reducing sugar of juice of healthy aril and seed was 41.79 mg/g of tissue FW and 6.50 mg/g of tissue FW respectively).

Changes in reducing sugar with increasing intensity of aril browning are presented in Fig.10. and Fig.11. The result showed that there was an increase in reducing sugar with increase in intensity of aril browning.The lowest reducing sugar for seed and juice from healthy aril was recorded as 6.11mg/g of tissue FW and 19.64mg/g of tissue FW respectively, which increases to 42.05 mg/g of tissue FW and 53.09 mg/g of tissue FW for seed and juice from high intensity of aril browning affected aril respectively.

4.6.2. Changes in total soluble solids with increasing intensity of aril browning

A significant increase in TSS was recorded from 14.4 % to 16.3 % for healthy aril and high intensity of browning affected arils respectively (Fig.12)

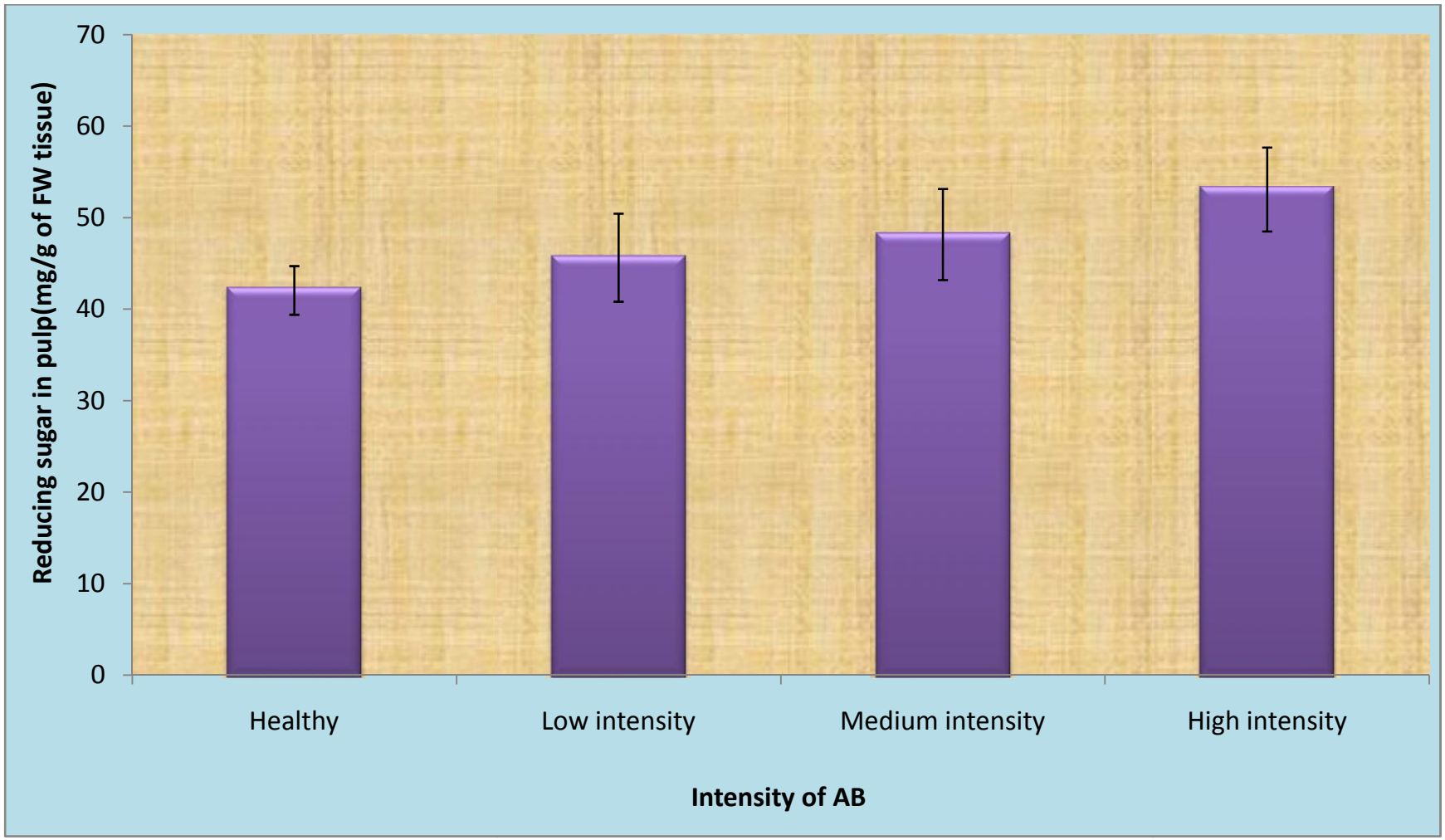


Fig. 10. Changes in reducing sugar in pulp with increasing intensity of AB

(I) **Vertical bars represent SE**

Fig. 11. Changes in reducing sugar in seed with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 6.696, SEm(±) 1.411)

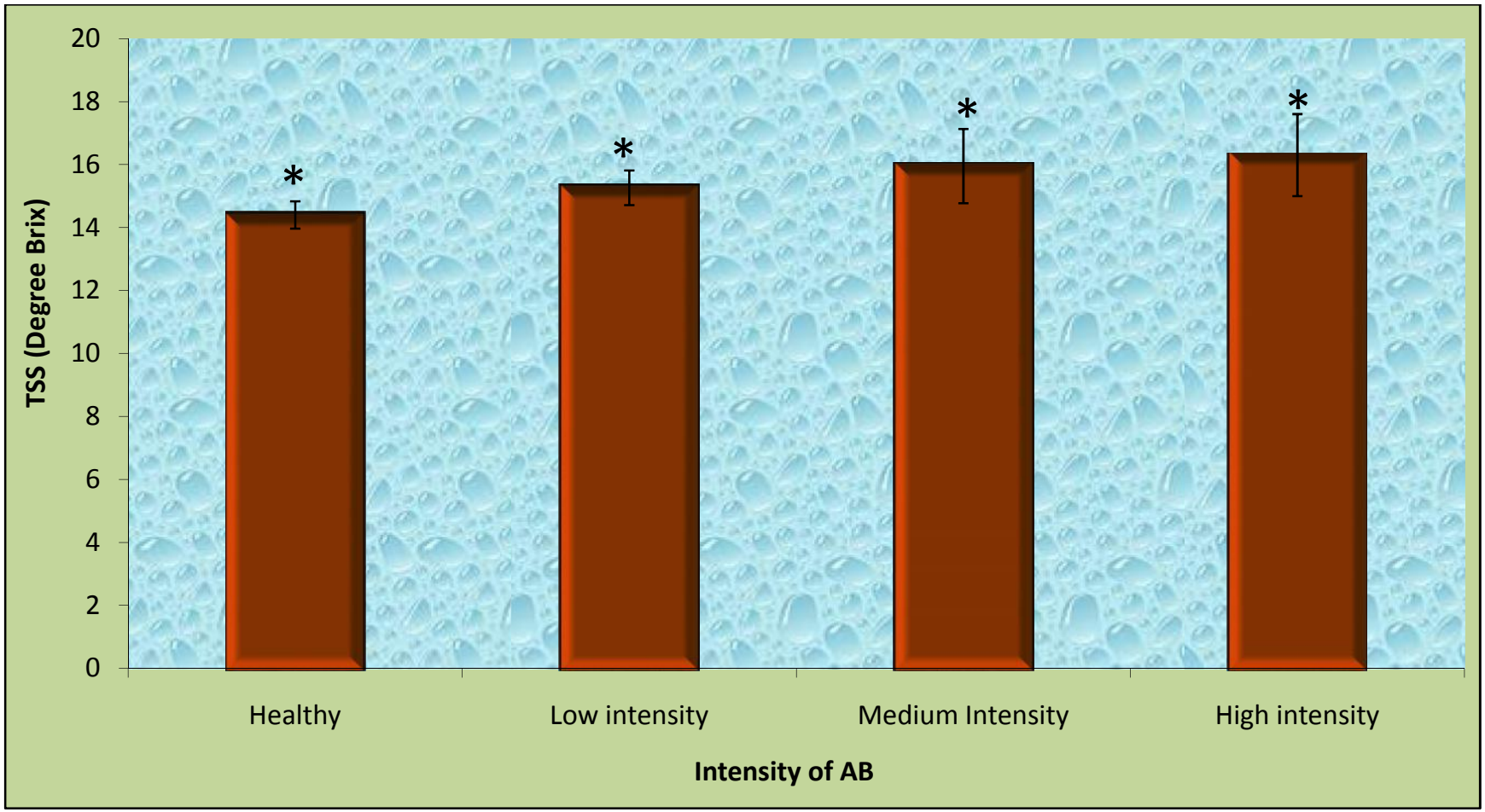


Fig. 12. Changes in TSS in juice with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at 5% (CD 5% = 1.762, SEm(±) 0.540)

Table 5. Total soluble sugars in healthy and aril browning affected aril fruit juice

Tissue status	Total Soluble Sugar (mg/g of tissue FW)[a]
Fruit juice from healthy arils	66.79
Fruit juice from AB affected arils	84.45
T-Test	**
T-Value	8.085

Table 6. Total soluble sugar in seed of healthy and aril browning affected aril

Tissue status	Total Soluble Sugar (mg/g of tissue FW)[a]
Seed of healthy aril	10.52
Seed of AB affected aril	29.89
T-Test	**
T-Value	20.378

a : average of five different observations

** Significant at 1% level

Table 7. Reducing sugars in healthy and aril browning affected aril fruit juice

Tissue status	Reducing Sugar (mg/g of tissue FW)[a]
Fruit juice from healthy arils	41.79
Fruit juice from AB affected arils	53.40
T- Test	**
T-Value	6.294

Table 8. Reducing sugar in seed of healthy and aril browning affected aril

Tissue status	Reducing Sugar (mg/g of tissue FW)[a]
Seed of healthy aril	6.50
Seed of AB affected aril	21.75
T-Test	**
T-Value	6.85

a : average of five different observations

**significant at 1% level

4.6.3. Starch content in the seed of healthy and aril browning affected aril

It is observed from Table 9 there was significant variation in the starch content of aril browning affected aril compared to healthy arils. Seed of healthy aril showed lower starch content (110.75 mg/g of tissue DW) compared to seed of aril browning affected aril (194.96 mg/g of tissue DW).

Changes in starch content with increasing intensity of aril browning are presented in Fig.13. The result showed that there was an increase in starch with increase in intensity of aril browning. The lowest starch for seed was recorded as 118.6 mg/g of tissue DW which increases to 200.77 mg/g of tissue DW for seed of high intensity of aril browning affected aril.

4.7. Total protein content in healthy and aril browned seed of pomegranate

Total protein content in the seed of aril browning affected aril and healthy seed is presented in Table 10. According to Table 10 the protein content in healthy seed was higher (7.832 mg/g of tissue FW) than the aril browning affected seed (6.902 mg/g of tissue FW).

4.8. Changes in titrable acidity (TA) and pH with increasing intensity of aril browning

The result of experiment on changes in titrable acidity revealed that there was significant decrease in TA content with increasing intensity of AB from 347 mg of citric acid/100g of aril for healthy aril to 161.07 mg of citric acid/100g of aril for high intensity of browning affected aril (Fig.14).

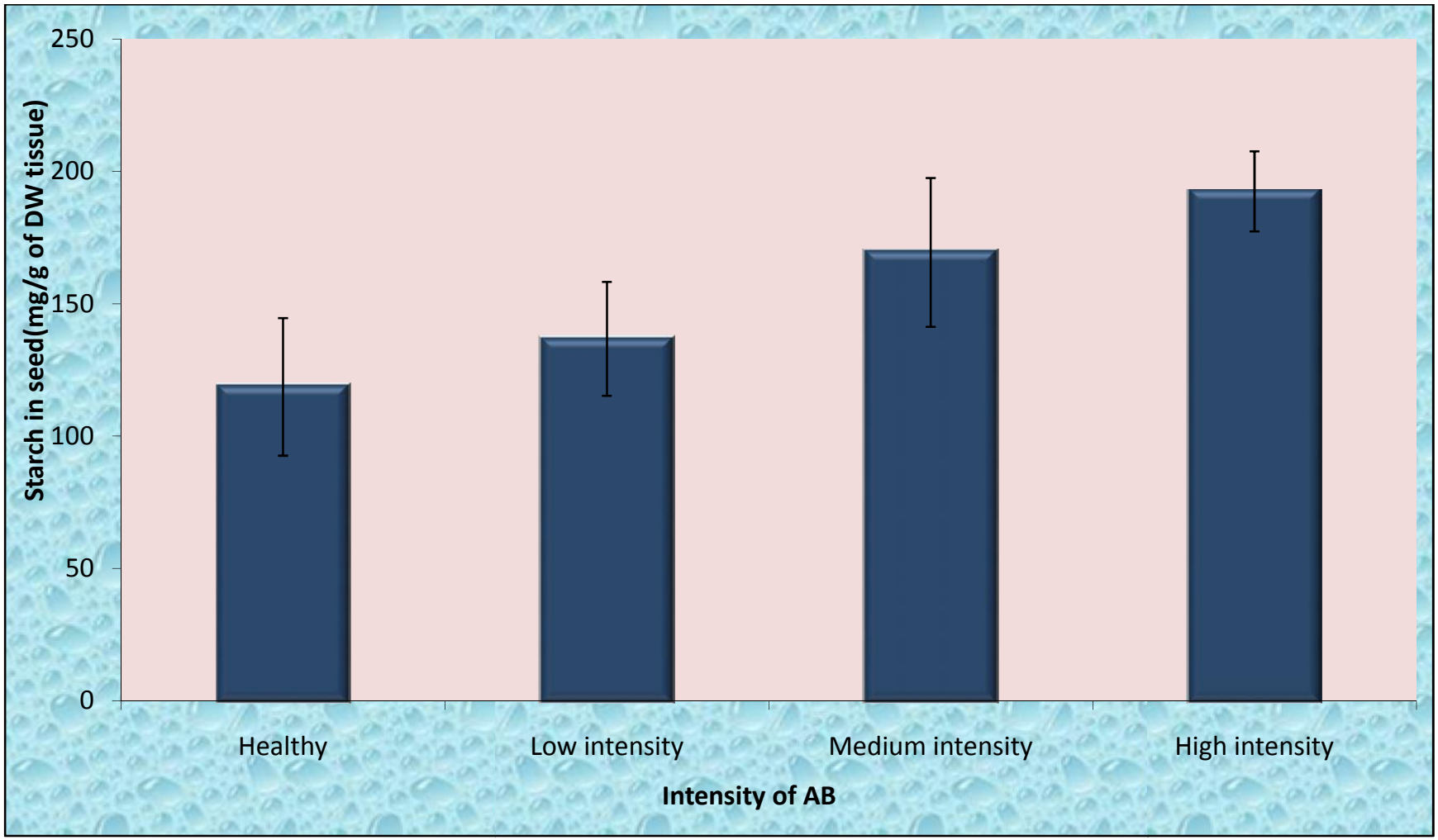


Fig. 13. Changes in starch content in seed with increasing intensity of AB

(I) **Vertical bars represent SE**

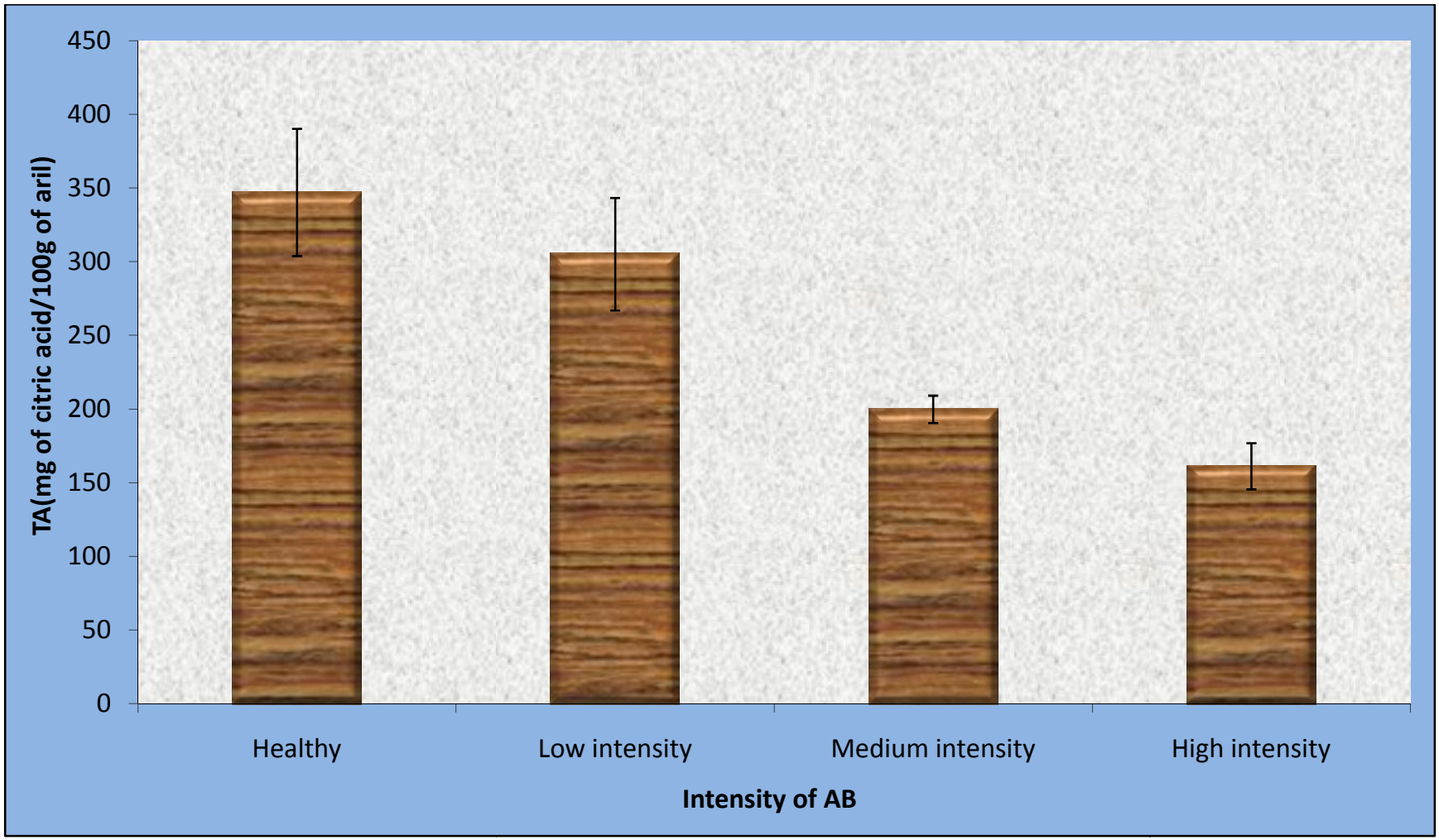


Fig. 14. Changes in titrable acidity (TA) with increasing intensity of AB

(I) **Vertical bars represent SE**

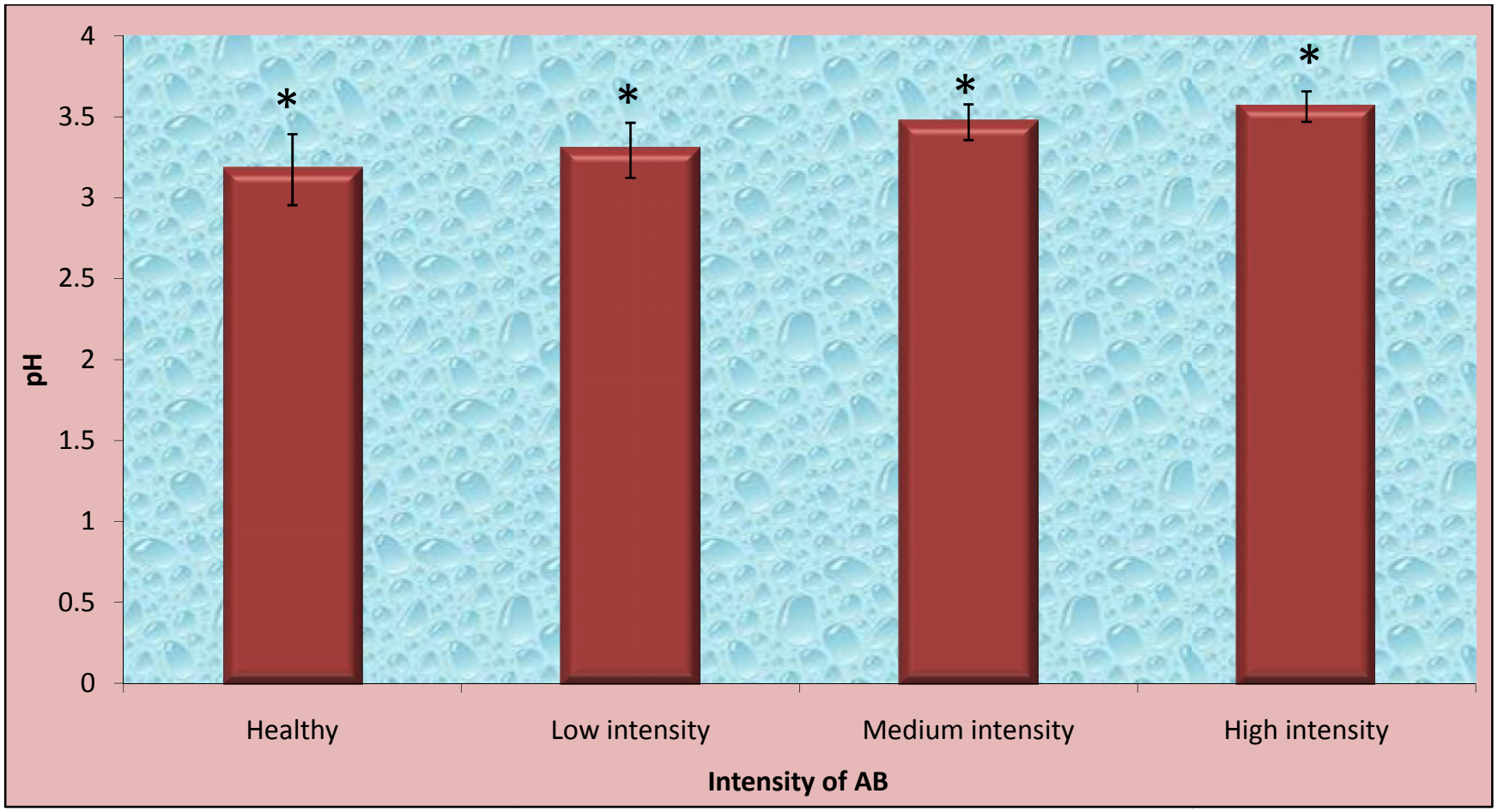


Fig. 15. Changes in pH with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 0.429, SEm(±) 0.0904)

Changes in pH with increasing intensity of aril browning are presented in Fig.15. The data indicated significant increase in pH with increasing intensity of AB from 3.10 for healthy to 3.56 for high intensity of browning affected aril.

Table 9. Starch content in seed of healthy and aril browning affected aril

Tissue status	Starch (mg/g of tissue FW)[a]
Seed of healthy aril	110.75
Seed of AB affected aril	194.96
T-Test	**
T-Value	4.974

Table 10. Total protein content in seed of healthy and aril browned affected aril

Tissue status	Total Protein (mg/g of tissue FW)[a]
Seed of healthy aril	7.832
Seed of AB affected aril	6.92
T-Test	**
T-Value	1.182

a : average of five different observations

**significant at 1% level

4.9. Changes in ascorbic acid content with increasing intensity of aril browning

Data on changes in ascorbic acid content with increasing intensity of aril browning showed that there was significant decrease in ascorbic acid content with the increase in intensity of aril browning from 9.76 mg/100g of aril for healthy aril to 4.64 mg/100g of aril for high intensity of browning affected aril (Fig.16).

4.10. Changes in anthocyanin content with increasing intensity of aril browning

Fig. 17. represents decrease in anthocyanin content with increasing intensity of aril browning. Results revealed that there was significant decrease in anothocyanin content with increase in intensity of aril browning from 0.531 OD changes/g of aril for healthy aril to 0.33 OD changes/g of aril for high intensity of browning affected aril.

4.11. Changes in total phenolics content with increasing intensity of aril browning

The reduction in total phenolics content with increasing intensity of aril browning is presented in Fig.18. Results reveals that there was significant decrease in total phenolics content with increase in intensity of aril browning from 141.25 mg/100g of aril for healthy aril to 109.76 mg/100g for high intensity of browning affected aril.

4.12. Changes in total dehydrogenase activity of seed with increasing intensity of aril browning

Dehydrogenase activity in seed of healthy and aril browning affected aril is presented in Table 11. The result indicated that the dehydrogenase activity was higher in seed of healthy aril (2.21 ΔA_{485}/g tissue FW) than the seed of AB affected aril (1.44 ΔA_{485}/g tissue FW).

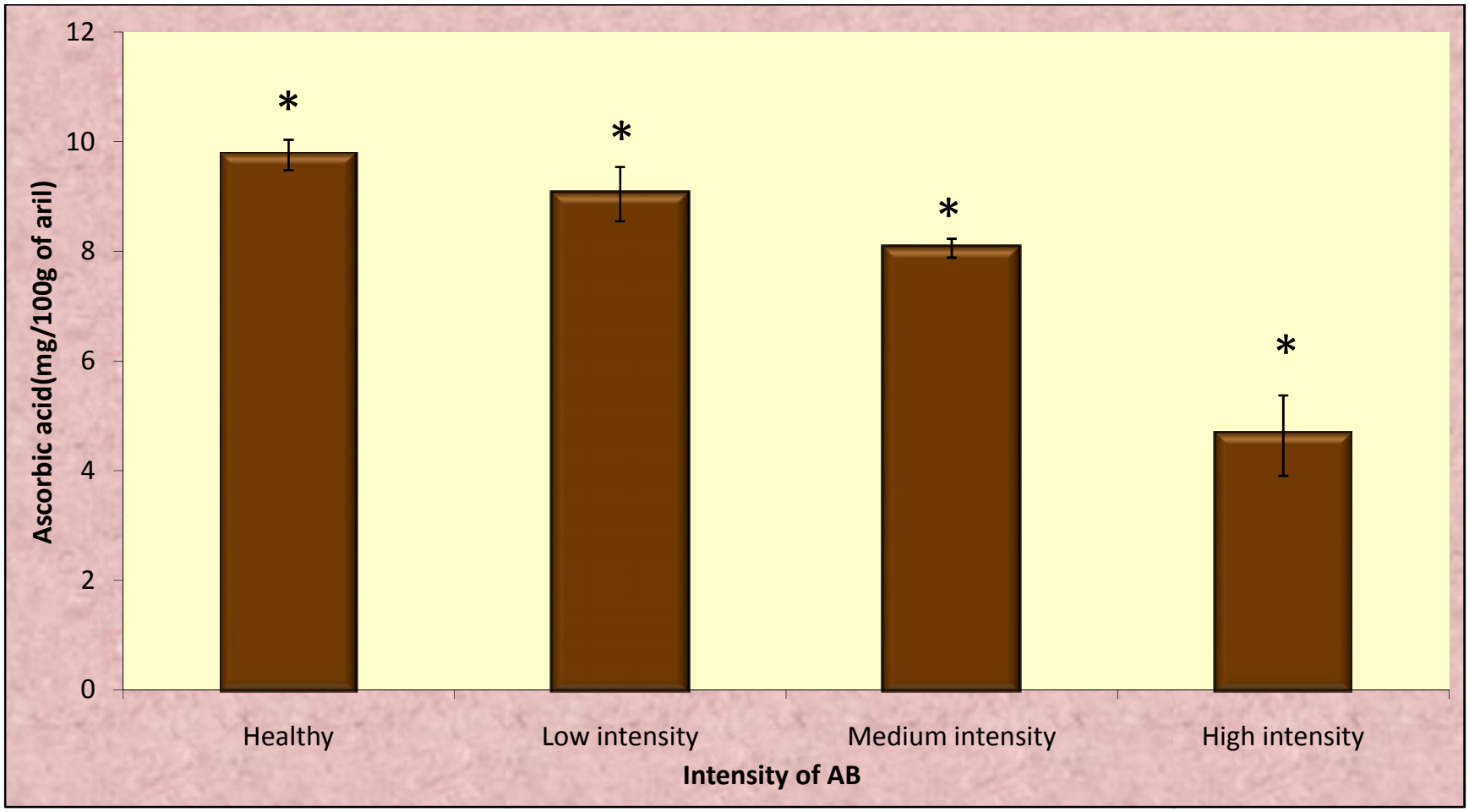


Fig. 16. Changes in ascorbic acid content with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 1.2925, SEm(±) 0.2724)

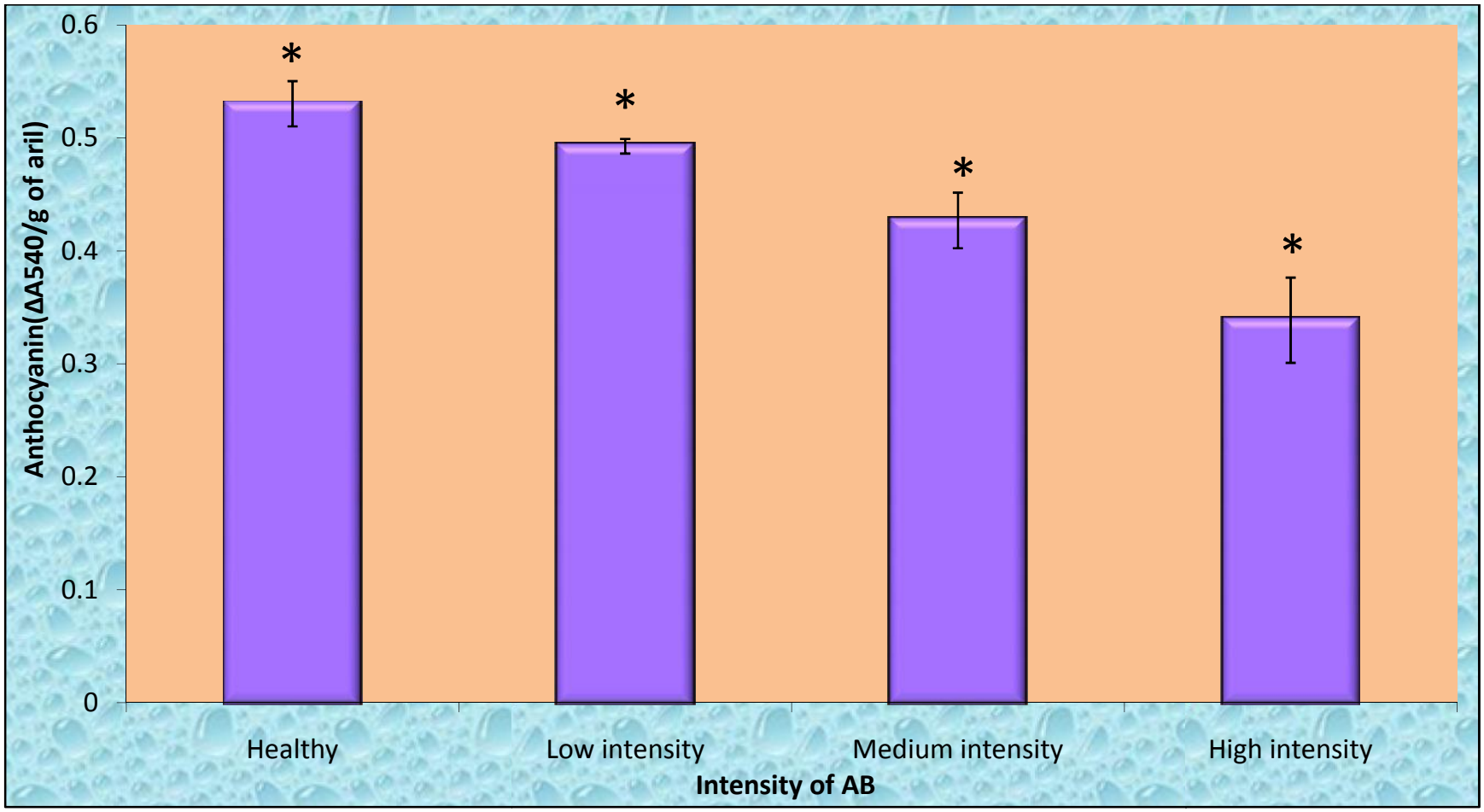


Fig. 17. Changes in anthocyanin content with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 0.0681, SEm(±) 0.0143)

Data regarding changes in dehydrogenase activity of seed with the increase in intensity of aril browning is presented in Fig.19. Seed of healthy aril showed highest activity (2.39 ΔA_{485}/g tissue FW) and high intensity of browning affected aril seed showed lowest activity (1.42 ΔA_{485}/g tissue FW).

4.13. Amylase activity in seed of healthy and aril browning affected aril

Amylase activity in the healthy and aril browning affected seed is presented in Table 12. The results revealed that amylase activity was almost four times higher in seed of healthy aril (7.36 mg maltose liberated/h/g of protein) as compared to the seed of aril browning affected aril (1.64 mg maltose liberated/h/g of protein).

4.14. Polyphenol oxidase activity in seed from healthy and aril browning affected aril

Polyphenol oxidase activity in the healthy and aril browning affected seed is presented in Table 13. The results showed that polyphenol oxidase activity was higher in seed of aril browning affected aril (0.0170 $\Delta A_{412} mg^{-1}$ protein min^{-1}) as compared to the healthy seed (0.0063 $\Delta A_{412} mg^{-1}$ protein min^{-1}).

4.15. Effect of preharvest plant growth regulator application on incidence of aril browning at different maturity

Table 14 represents effect of preharvest treatment on incidence of aril browning. Results showed that preharvest treatment of gibberellic acid (GA_3) and paclobutrazol (PBZ) affects the incidence of aril browning. Fruits treated with GA_3 showed less incidence(2 arils at 70% maturity, 4 arils at 80% and 7 arils at 90% maturity per 100 arils examined) as compared to control (6 arils were affected with AB at 70%, 9 arils at 80% and 16 arils at 90 % were affected with AB per 100 arils examined) at all

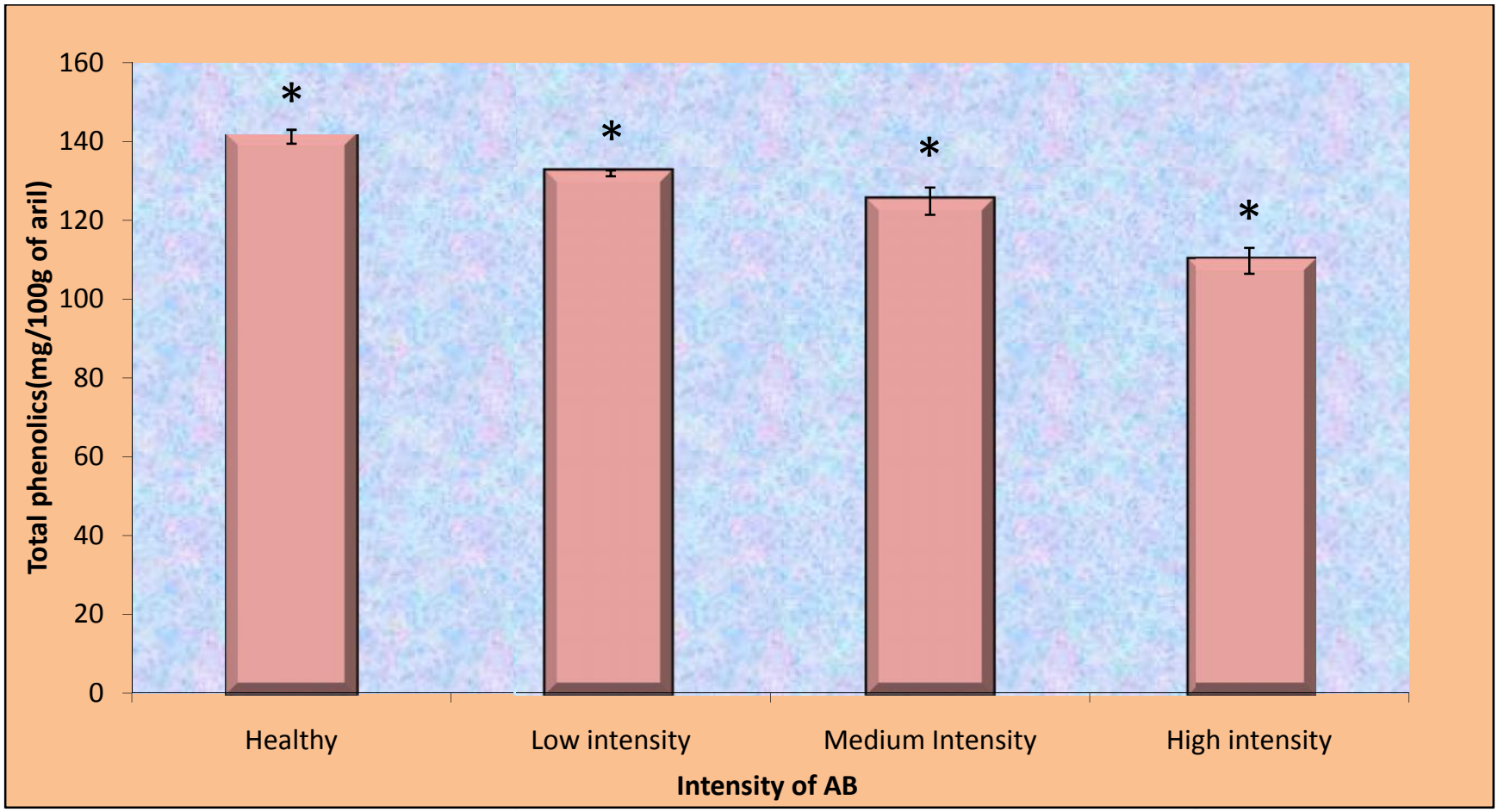


Fig. 18. Changes in total phenolics content with increasing intensity of AB

(⌶) **Vertical bars represent SE**

Significant at1% (CD 1% = 7.076, SEm(±) 1.491)

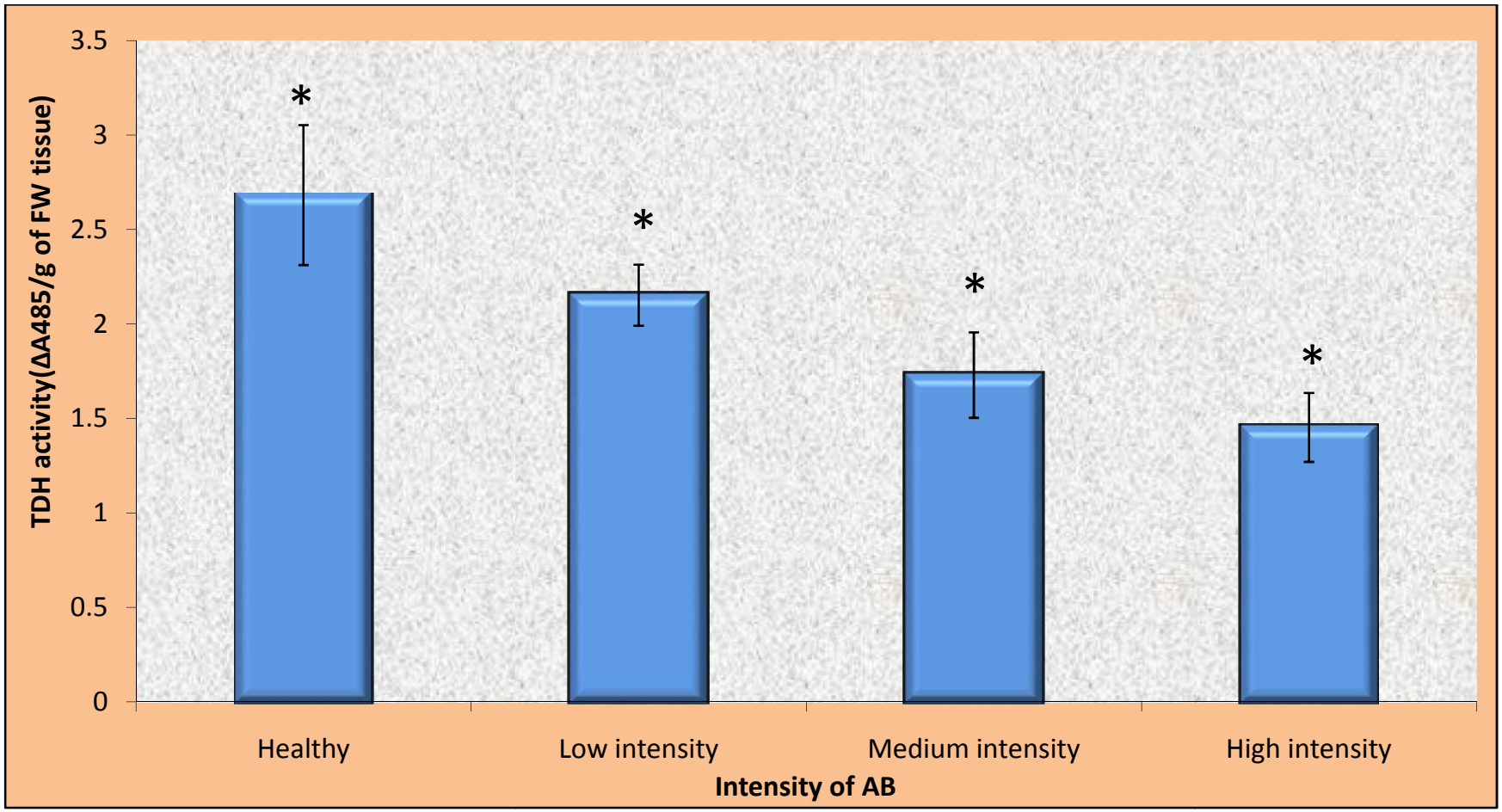


Fig. 19. Changes in TDH activity with increasing intensity of AB

(I) **Vertical bars represent SE**

Significant at1% (CD 1% = 0.681, SEm(±) 0.1437)

stages of maturity while PBZ treated fruits showed more incidence(9 arils at 70%, 15 arils at 80% and 20 arils at 90% were affected with AB per 100 arils examined) as compared to control at all maturity. Results also represented that there was an increase in incidence in CaCl2 treated fruits (8 arils at 70%, 11 arils at 80% and 20 arils at 90% were affected with AB per 100 arils examined) as compared to control fruits.

Table 11. Dehydrogenase activity in seed of healthy and aril browning affected aril

Tissue status	Dehyrogenase activity (ΔA_{485}/g tissue FW)[a]
Seed of healthy aril	2.21
Seed of aril browning affected aril	1.44
T-Test	**
T-Value	5.632

Table 12. Amylase activity in healthy and aril browned seed of pomegranate

Tissue status	Amylase activity (mg maltose liberated/h/g of protein)[a]
Seed of healthy aril	7.36
Seed of aril browning affected aril	1.64
T-Test	**
T-Value	5.64

a : average of four different observations

**significant at 1% level

Table 13. Polyphenol oxidase activity in healthy and aril browned seed of pomegranate

Tissue status	Polyphenol oxidase activity ($\Delta A_{412} mg^{-1} protein\ min^{-1}$)[a]
Seed of healthy aril	0.0063
Seed of aril browning affected aril	0.0170
T-Test	**
T-Value	4.984

a : average of four different observations

**significant at 1% level

Table 14. Intensity of browning in fruits treated with growth hormones and $CaCl_2$

(a) At 70% maturity

	No. of arils affected with AB per 100 arils examined			
	Control Fruit	**GA_3 treated fruit**	**PBZ treated fruit**	**$CaCl_2$ treated fruit**
Big spot	6	2	9	8
High intensity	_	_	_	_
F-test	**			
CD value at 1%	2.01			
SEm(±)	0.519			

**Significant at 1%

(b) At 80% maturity

	No. of arils affected with AB per 100 arils examined			
	Control Fruit	**GA_3 treated fruit**	**PBZ treated fruit**	**$CaCl_2$ treated fruit**
Big spot	8	4	14	11
High intensity	1	_	1	_
F-test	**			
CD value at 1%	3.74			
SEm(±)	0.966			

(c) At 90% maturity

	No. of arils affected with AB per 100 arils examined			
	Control Fruit	**GA_3 treated fruit**	**PBZ treated fruit**	**$CaCl_2$ treated fruit**
Big spot	15	7	20	20
High intensity	1	_	2	_
F-test	**			
CD value at 1%	3.28			
SEm(±)	0.84			

**Significant at 1%

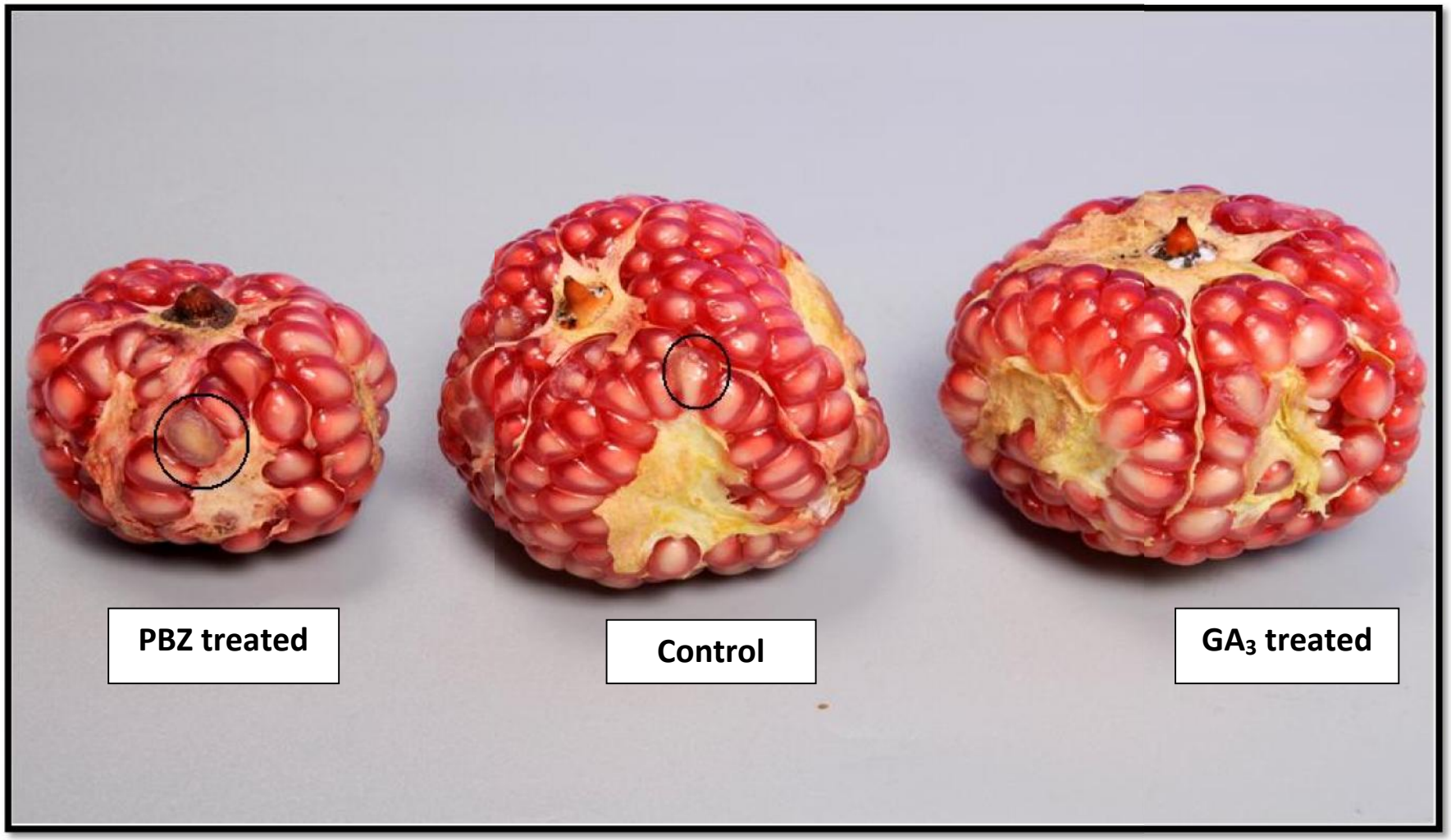


Fig. 20. Fruits showing differences in incidence of browning with respect to different treatment

Fig. 21. Fruit showing incidence of AB as first observed near the calyx end just under the skin

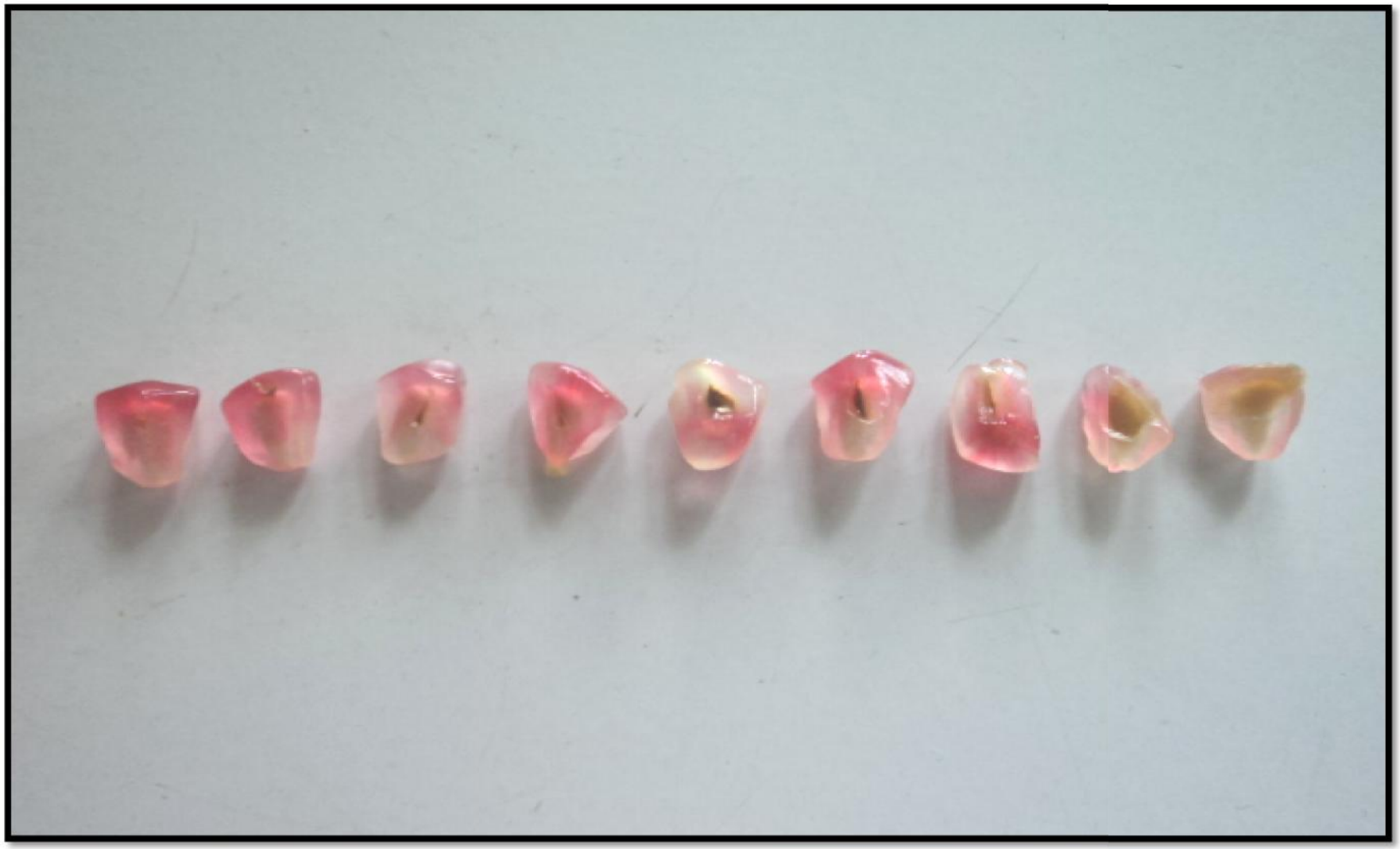


Fig. 22. Arils showing increasing intensity of browning

Fig. 23. Differences in seed of healthy aril and aril browning affected arils

Discussion

V. DISCUSSION

The present study was undertaken with the main objective of understanding the factors affecting the incidence of aril browning in pomegranate and the biochemical changes occurring during development of disorder, effect of plant growth regulators on aril browning incidence, role of seed and its relation to incidence of aril browning and effect of fruit location, fruit maturity and fruit number on incidence of aril browning.

5.1.1. Effect of fruit location on the incidence of aril browning

Table 3a. indicated that the fruits exposed to sun showed less aril browning as compared to fruits inside canopy. Zerbini *et al* (2002) reported that 'Conference' pears grown in the Mediterranean area are less susceptible to browning disorder than pear grown in the northwest Europe. They explained that exposure to sunlight increases the ascorbic acid content of fruit which helped in reducing browning. In our experiments, it was observed that ascorbic acid content in healthy arils was higher than that found in browned arils as shown in fig. 16. From this, it was apparent that the higher ascorbic content in sun exposed fruits could be responsible for the lower incidence of AB as compared to those fruits which were under shade. Sawant (1993) found that spraying of ascorbic acid on the pomegranate plants as an antioxidant showed a significant effect on all biochemical parameters of blackening suggesting that ascorbic acid may be playing a role in induction of aril browning in pomegranate.

Results presented in Table 3b. showed differences in incidence of aril browning due to fruit location on main shoot and lateral shoot. The incidence of aril browning was more in case of fruits located on lateral shoots as compared to fruits located on main shoot. Smock (1914)

observed in apple that the central fruit on the spur was less susceptible to bitter pit than the lateral fruits. This could be explained on the basis of competition for nutrients. Fruits located on main shoot are nearer to source and get a better nutrient supply as compared to fruits located on lateral shoots.

5.2. Influence of fruit load on the incidence of aril browning

Table 4 indicated that the aril browning incidence was more in case of panicles having two fruits as compared to panicle with only one fruit. These results revealed that the inter fruit competition may be playing a role in induction of aril browning in pomegranate.

5.3. Effect of maturity on the incidence of aril browning

Result of Figure 4 showed that incidence of aril browning increased with increasing maturity. This result indicated that the delayed harvesting of fruit might be the one of the possible contributing factor for the high incidence of aril browning in pomegranate. Subramanyan *et al.* (1971) observed that fruits with breakdown were at optimum stage of maturity. Limaye *et al.* (1976) studied the occurrence of spongy tissue in relation to stage of maturity at harvest. They found that a low proportion (10.5%) of fruits were affected by spongy tissue when harvested at 'A' stage (12 anna), and the maximum incidence (87.6%) was observed in fruits harvested at 'D' (tree ripe) stage. If the fruits were harvested at 'B' stage of maturity (14 anna), the occurrence of spongy tissue was considerably low (23.33%) and the fruit quality of ripe fruits were less affected than those harvested at the fully ripe stage. Similar observation was made by Joshi (1975) and Joshi and Limaye (1984).

5.4. Differences in germinability between seed from healthy and aril browning affected arils

Results presented in Figure 5 showed the relationship between percent germination of seeds from healthy and AB affected arils. The results showed that the seed from healthy arils took less time for germination than the seed from AB affected arils. Result also showed that the germination percentage of seed from healthy was higher (92%) than the seed from AB affected aril (74 %). Ravindra and Shivashankar (2004) found that there was a considerable difference in the number of days taken for germination between seeds from spongy tissue affected and healthy fruit. According to their experiment, the affected fruit seed took less number of days for germination than the healthy fruit seed. In case of pomegranate, seed from AB affected aril took more time and germination percentage of seed from AB affected aril seed was less than seed of healthy aril. This indicated that reduced seed viability might play a role in the development of AB.

5.5. Biochemical changes occurring during disorder development

5.5.1. Moisture content

Healthy and AB affected aril showed significant differences with respect to moisture content both in seed and pulp. Moisture content of both pulp and seed was higher in case of healthy aril as shown in Fig 6 and Fig 7. The decrease in moisture content of both pulp and seed of aril browning affected aril indicated the mobilization of water away from the arils. Similar results of decrease in moisture content of spongy tissue affected mango fruits mesocarp was observed by Shivashankar *et al.* (2007).

5.5.2. Changes in total sugar and reducing sugar content

Results presented in Table 5, Table 6, Table 7 and Table 8 showed differences in the levels of total sugar and reducing sugar in healthy and aril browning affected aril respectively. There was an increase in levels of both total sugar and reducing sugar in both juice and seed from AB affected aril. This was also further supported by the observation that total sugar and reducing sugar levels in both juice and seed increased with the increase in intensity of aril browning as compared to juice and seed of healthy aril (Fig. 8, Fig. 9, Fig. 10 and Fig. 11). The data of changes in TSS in juice with increasing intensity of AB also showed a similar trend as shown in Fig. 12. The increase in total sugar, reducing sugar and TSS in browning affected arils could be due to decreased moisture content in browning affected arils as compared to healthy aril (Fig.6 and Fig.7). Tobar *et al.* (2009) reported that the peel percent, dry matter of juice, acidity, total soluble solids and total sugars increased faster in case of disorder fruit(aril browned fruit) than those in intact fruit(healthy fruit). The increase in total sugar could be explained on the basis of observation by Zhang *et al.*(2005). During the process of anthocyanin degradation sugar moiety acts as steric hindrance to PPO (Polyphenol Oxidase) attack (Zhang *et al*, 2001). Zhang *et al.* (2005) indicated that the anthocyanase could first remove the sugar moiety from litchi anthocyanins, producing anthocyanidins, and finally POD causes the degradation of the anthocyanidin in the presence of H_2O_2. Similar phenomenon may be involved in aril browning in pomegranate and this sugar moiety separated from anthocyanin ring may contribute to the increased sugar concentration in AB affected arils as compared to healthy aril. This is further supported by increase in sugar concentration with the increase in intensity of AB as shown in Fig.7, Fig. 8 , Fig. 9 and Fig.10)

5.5.3. Changes in Starch content

Starch content of AB affected arils was significantly higher as compared to healthy arils (Table 9). These results are in agreement with those reported by Shivashankar *et al.* (2006) in pomegranate, Rangawala (1975), Patkar (1978), Katrodia (1979) and Shivashankar *et al* (2007) . This was further supported by the observation that the starch content in seed increased with the increase in intensity of aril browning as compared to seed of healthy aril (Figure 13). Shivashankar *et al.* (2004) suggested that the browning of arils in pomegranate resulted in lower starch and acid metabolism.

5.5.4. Amylase activity

The results in Table 12 revealed that amylase activity in seed from AB affected aril was less than seed of healthy aril. This result when analysed along with the data of Table 9 showed that the lower starch content of healthy seed could be due to higher amylase activity in seed from healthy aril as compared to seed from AB affected aril. It is likely that the lower activity of amylase in seed from AB affected aril could be the result of decreased moisture content of seed from AB affected aril as against the seed from healthy aril.

5.5.5 Total protein content

There was higher total protein content in the seed of healthy arils than the seeds from AB affected arils (Table10). Gupta *et al.* (1985) and Shivashankar *et al.* (2007) observed difference in total protein content in affected and healthy mesocarp of spongy tissue affected fruit. Decrease in total protein content of seeds from AB affected arils indicates that there was decrease in metabolic activity of seeds which leads to non viability of seeds from AB affected arils.

5.5.6. Total Dehydrogenase activity

The total dehydrogenase activity was more in case of seed from healthy aril as compared to seed from AB affected aril (Table 11). The total dehydrogenase activity of seed decreased with the increase in intensity of aril browning (Fig. 19).These results supported the lower germination rate and viability of seed as shown in Fig.5. Maeprasart *et al.* (1999) reported a direct positive correlation between seed dehydrogenase activity and seed viability. The observed low dehydrogenase activity in seed from AB affected aril supports the decreased viability of seed from AB affected aril as against the seed from healthy aril. It is likely that the lower dehydrogenase activity in seed from AB affected aril could be the result of decreased moisture content of seed from AB affected aril as against the seed of healthy aril.

5.5.7. Change in pH and Titrable acidity

Results presented in Figure 15. showed a gradual increase in pH with increase in intensity of AB from 3.10 to 3.56. This was further supported by the observation that there was gradual decrease in titrable acidity with the increase in intensity of aril browning (Fig 14). Decrease in titrable acidity leads to increase in pH.

5.5.8. Changes in Polyphenol Oxidase (PPO) activity

Results presented in Table 13 showed that seed from AB affected aril showed more PPO activity than seed from healthy aril. This was further supported by the observation that there was higher pH in case of AB affected arils (Fig. 15) and PPO is more active at higher pH.

5.5.9. Changes in Anthocyanins

There was a gradual decrease in anthocyanin content with the increased intensity of AB (Fig. 17). Decrease in anthocyanin content of

the aril could be explained on the basis of browning mechanism in other fruits like litchi. Post harvest browning of litchi was thought to be caused by a rapid degradation of the red pigment by polyphenol oxidase (PPO), producing brown coloured by-product (Akamine, 1960; Huang *et al.*,1990). Recently Jiang (2000) reported that litchi PPO cannot oxidize anthocyanin, but the anthocyanin might be degraded rapidly in an anthocyanin – PPO - phenol system and, thus, suggested that it may be the presence of the sugar moiety which caused steric hindrance. Since anthocyanins are unstable; they could be degraded nonenzymatically or enzymatically. In addition, anthocyanase (anthocyanin-β-glucosidase) could play a role in removing the sugar groups, leading to the anthocyanin decolourization (Huang *et al.*, 1990). This was further supported by results of PPO activity which showed an increase in PPO activity in seed of AB affected aril as compared to seed of healthy aril.

5.5.10. Changes in ascorbic acid content

Results presented in figure 16. showed that there was a decrease in ascorbic acid content with the increase in aril browning intensity. Similar results observed by Noctor and Foyer(1998) in pear, 'Conference' pears tend to develop tissue disorders, like brown core, when ascorbic acid levels drop below a certain value. Decline in ascorbate concentrations is often associated with reduced capability to prevent oxidative damage. Sawant (1993) found that the spraying of ascorbic acid on the pomegranate plants as an antioxidant showed a significant effect on all biochemical parameters of blackening.

5.5.11. Changes in total phenolics

There was a gradual decrease in total phenolics content with the increased incidence of aril browning (Fig. 18). This may happen due to higher activity of PPO which causes oxidation of phenols.

5.6. Treatment of fruits with plant growth regulators and $CaCl_2$

Results indicated that (Table14(a), (b) and(c)) fruits treated with GA_3 showed less incidence of AB as compared to control and fruits treated with PBZ showed more incidence of AB as compared to control. Treatment of fruits with $CaCl_2$ also showed increased incidence of AB as compared to control. Treatment of fruits with GA_3 enhances the sink strength of the fruits which reduces AB incidence while PBZ which act as inhibitor of GA_3 activity reduces sink strength which leads to development of more browning. PBZ treated fruits also showed high intensity of browning as compared to control and GA_3 showed less intensity of browning as compared to control (Fig.20).

The above data based on alteration of sink strength by external application of plant growth regulators clearly shows that the incidence of browning in pomegranate might be related to the sink capacity of the fruit.

Summary

VI. SUMMARY

The salient points of the study are summarized as under:-

- The incidence of aril browning varied in direct proportion with the number of fruits per panicle. Results revealed that AB incidence arises may be due to inter fruit competition. Due to interfruit competition, sink of variable strength are developed leading to differences in the supply of nutrients and water to developing fruit.
- The incidence of aril browning in cv. Bhagwa was first observed at 50 % maturity (70 days from the fruit set).
- Incidence of aril browning in arils was initially found to occur in the lower part of the fruit (near to the calyx end) and just under the skin (peel)(Fig.21).
- Browning starts as a small greyish white dot on the aril which increases in diameter during fruit maturation. When the spots attain a diameter of 1-1.5 mm, browning occurs and the arils show a shrivelled appearance with a brown to black colour (Fig.22).
- In advanced stage of browning black scar was observed on the seed of aril browning affected aril (Fig.23).
- During the development of the fruit disorder, the levels of
  - Total sugar
  - Reducing sugars
  - TSS (Total Soluble Solids)
  - Starch
  - pH, were found to increase in AB affected aril as compared to healthy aril.

- Amylase activity was found to decrease with increasing AB development resulting in higher levels of starch.
- During the development of the fruit disorder, the levels of
  - Titrable acidity
  - Anthocyanins
  - Total phenolics
  - Ascorbic acid, were found to decrease in AB affected aril as compared to healthy aril.
- Increase in pH with the increasing intensity of AB was confirmed by the data on titrable acidity, which decreased with the increase in intensity of AB.
- PPO activity increased with increasing intensity of AB associated with a concomitant reduction in the levels of polyphenols.
- Moisture content in seed and pulp of healthy aril was higher in comparison with that of seed and pulp from AB affected aril.
- Experiment conducted on the germination of seed from healthy and AB affected aril showed that the percent and rate of germination in healthy seed was higher as compared to AB seed indicating that there was a reduction in viability of seeds from AB affected arils.
- Total dehydrogenase activity in the seed was less in case of seed from AB affected arils as compared to seed from healthy aril confirming that the seed viability was reduced in AB affected seed.
- The above results clearly indicated that loss of moisture from seed from AB affected aril resulted in reduction in seed viability.

- Results of experiment on the application of plant growth regulators indicated that increase of sink strength by GA3 treatment resulted in decreased incidence of AB and decrease of sink strength by PBZ treatment resulted in increased incidence of AB.

In summary, the findings of the present study indicated that interfruit competition among the fruits growing at the same time leads to development of sinks of different strength depending on their location. Such differences results in unequal distribution of nutrients to developing fruits which disturbs the physiology of fruit development leading to biochemical changes which ultimately leads to initiation of aril browning which once begun is irreversible.

Future line of work

1. Development of aril browning in pomegranate is a complex process. So there is need to understand the biochemical mechanism of browning during development of the disorder.

2. Studies on aril browning susceptible and resistant varieties during fruit ontogeny must be carried out in order to understand the basic difference which leads to development of the disorder.

3. Studies to develop appropriate control measures for the successful management of aril browning in commercial varieties which are highly susceptible to aril browning.

References

VII. REFERENCES

AKAMINE, E.K., 1960, Preventing the darkening of fresh lychees prepared for transport. *Technical Programme Report. Hawaii Agricultural Experimental Station. University of Hawaii, No. 127*.

ALI, A.M., AND KELLY, W.C., 1992, The effect of interfruit competition on the size of sweet pepper (*Capsicum annuum* L.) fruits. *Sci. Hort.*, **52:** 69-76.

AMIN, H.D., PANDAYA, H.G. AND BHUVA, H.D.,1974, Varietal status of mango in Gujrat. *Paper presented at First All India Seminar on mango and mango culture.* pp.1-7.

ANET, E.F., AND COGGIOLA, J.M.,1974, ,Superficial scald, a functional disorder of stored apples. *J. Sci. Food agri.*, **25**: 293-298.

AOAC, 1965, *Official methods of analysis* : 10th edn (Washington, Association of Analytical Chemists).

AOAC, 1970, *Official methods of analysis* : 11th edn (Washington, Association of Analytical Chemists).

BASHIR, H.A., AND ABU-GOUKH, A.B.A., 2003, Compositional changes during guava fruit ripening. *Food Chem* ., **80** :557-563.

BEN, J.,1986, The effect of Calcium compound storability of Jonathan apples. Part I. Effect of spraying the trees with calcium chloride and calcium chelate on the occurrence of characteristics. *Acta Agraria et silvestrica agaria.,* **25**:119-129.

BERGH, O., FRANKEN, J., ZYL, E.J., VAN, K.F., AND DEMPERS, A., 1980, Sunburn on apples – preliminary results of an investigation conducted during the 1978/79 season .*Deciduous fruit Grower.*, **30**: 8-22.

BERNFEILD, P., 1955, Amylase, In: *Methods in Enzymology,* **Vol. I**., (Colowick, S.P. and Kapalan , S. Eds.)pp.149.

BIAIE, J.B., 1960, The postharvest biochemistry of tropical and subtropical fruits . *Adv. Food Res.,* **10** : 293-354.

BRAMLAGE, W.J., WEIS, S.A., AND DRAKE, M., 1985, Predicting the occurrence of post storage disorder of McIntosh apples from preharvest mineral analysis. *J.Amer. Soc. Hort. Sci.*, **110**(4): 493-498.

BRENNER, M.L., AND CHEIKH, N., 1995, The role of hormones in photosynthate partitioning and seed filling. In: Davies PJ, editor. Plant hormones: physiology, biochemistry, and molecular biology. 2nd ed. Dordrecht, *The Netherlands: Klewer Academic Publishers*, pp 649–670.

CHEEMA, G.S., AND DANI, P.G., 1934, Report on the export of mangoes to Europe in 1932 and 1933. *Dept agric. bombay ,Bull no*. **170**:pp. 1-31.

COOK, M.G. AND EVANS, L.T., 1978, Effect of relative size and distance of competing sink on the distribution of photosynthetic assimilates in wheat. *Aust.J.Plant Physiol.*, **5**: 493-509.

CRANE, J., 1964, Growth substances in fruit setting and development. *Annu Rev Plant Physiol* .,**15** :303–326.

CRISOSTO, C. H., MITCHELL, F. G., AND JOHNSON, R. S., 1995, Factors in fresh market stone fruit quality . *Postharvest News and Info.*, **6**(2) : 17-21.

CURRY, E.A., 1988, Chemical control of vegetative growth of Delicious fruit trees with paclobutrazol and Rsvio 411. *Hort Sci.*, **23**: 470-473.

DEFILIPP,B.G., BRUCE, D., BETTY, M., HESS, P., AND KADER, A. A., 2006, Development and control of scald on wonderful pomegranates during long-term storage., *Postharvest Biol.& Tech.,* **41**(3): 234-243 .

DELVER ,P., 1980, Calcium sprays against bitter pit and internal breakdown. *Fruiteelt.*,**70** (28) : 876-877.

DESAI, P.V.G.,1989, Investigation on internal breakdown of pomegranate fruit *Ph.D thesis submitted to Mahatma Phule Agriculture University ,Rahuri, India.*

EGLI, D.B.,1994, Seed growth and development .In : Boote KJ, Bennet JM, Sinclair T, Paulsen GM (eds) , Physiology and determination of crop yield, *Crop Sci. Soc. of Am. , Madison*, pp 127-147

ESTERBANER, H., SCHWAEZL, E., AND HAYN, M., 1977, *Anal Biochem.*,**77** : 486.

EUWENS, C. J., AND SCHWABE, W.W., 1975, Seed and pod wall developments in *Pisum sativum* L. in relation to extracted and applied hormones. *J.Exp.Bot.* **26**:1-14.

ESKIN, N.A.M., 1979, Plant pigment, Flavours and Textures: The chemistry and Biochemistry of selected compounds. Academic Press, New York.

FALLAHI, E., CONWAY, W.S., HICKEY,K.D., AND SAMS, C.E.,1997, The role of calcium and nitrogen in postharvest quality and disease resistance of apples. *HortSci.*, **32**:831-835.

FANKHAUSER, F., SCHUMACHER, R., AND STADLER, W., 1979, Application of calcium chloride by air blast spraying and in combination with fungicides. *Schwiezerische Zeitschrift fiir obst. Und Weinban* .,**115** (17): 550- 553.

FAUST, M., SHEAR, C.B., AND WILLIAMS, M.W.,1969,Disorder of carbohydrate metabolism of apples. *Bot. Rev.*, **35**:169-194.

FRANKHAUSER, F., AND STADLER, W.,1981, Care is needed when using high concentration calcium chloride application. *Schwiezerische Zeitschrift fiir obst. Und Weinban.*,**117**(19) : 545-547.

FERGUSON, I.B., AND WATKINS, C.B., 1983, Cation distribution and balance in apple fruits in relation to calcium treatment for bitter pit. *Scientia Hortic* . **19** (3-4):301-310.

FERGUSON, I.B., AND WATKINS, C.B.,1989, Bitter pit in apple fruit. *Hortic. Rev.*, **11**: 289-355.

FILSOWF, F.,1988, Chemicals affecting development of water core in apple fruit. *Iranian J. Plant Pathology.*, **24**(1-4): 21-23.

FORD, E.M., 1979, Effect of post-bloom environmental condition on fruit composition and quality of apples. *Commun. Soil Sci. Plant Anal.*, **10**: 337-348.

FRANCK, C., LAMMERTYN, J., TRI, H.O.,VERBOVEN, Q., VERLINDEN, P., AND NICOLAI,B.M., 2007, Browning disorder in pear fruit. *Postharvest Biol. And Techol.*, **43**:1-13.

GARMAN, P., AND MATHIS, W.J., 1956, Studies of mineral balance as related to occurrence of Baldwin Spot in Connecticut. *Connecticut Agricultural Experiment Station,Bull No.* **601**.

GRANT, J.A., AND MITCHAM, E.J., 1995, Internal browning of Fuji : Central California experiences . Proceedings of 11th Annual Washington .*Tree fruit Postharvest conference, Washington*, USA.

GILLASPY,G., BEN-DAVID, H., AND GRUISSEM, W., 1993, Fruits:a developmental perspective. *Plant Cell.*, **5**:1439–1451.

GRAEBE, J. E., 1987, Gibberillin biosynthesis and control. *Ann. Rev. Pl. Physiol.* **38**: 419- 465.

GUPTA, D.N., LAD, B.L., CHAVAN, A.S., AND SALVI, M.J., 1985, Enzyme studies on spongy tissue : A physiological ripening disorder in Alphonso mango. *J. Maharashtra Agric. Univ.,***10** : 280-282.

GUNJATE, R.T., TARE, S.J., RANGWALA, A.D., AND LIMAYE, V.P., 1979, Effect of preharvest and postharvest calcium treatment on calcium content and occurrence of spongy tissue in Alphonso mangoes. *Indian J.Hort,* **36**: 140-144.

HARTMAN, J.F., 1983, Calcium sprays: a pre – condition for good quality. *Fruit let.,* **73** (30): 797.

HEDDEN, P. AND HOAD, G. V., 1985, Hormonal regulation of fruit growth and development. Regulation of sources and sinks of crop plants. *Bristol: British Plant Growth Regulator Group*, *Monograph,* **12**: 211-221.

HENDRY, G.A.F. AND HOUGTON, J.D., 1996. Natural food colorants. Blackie Academic & Professional, an Impart of Chapman & Hal1, London, UK.

HEUVELINK, E., AND BUISKOOL, R.P.M., 1995, Influence of sink – source interaction on dry matter production in tomato, *Anal of Bot.*, **75**:381-390.

HUANG, S., HART, H., LEE, H., AND WICKER, L., 1990, Enzymatic and colour changes during post harvest of lychee fruit. *J. Food Sci.,* **55**: 1762- 1763.

HURD, R.G., GRAY A.P., AND MOUNTIFIELD, A.C., 1979, The effect of partial flower removal on the relation between roots, shoots and fruit grown in the indeterminate tomato. *Ann. of Appl. Biol.* **93**: 77-89.

INDIAN HORTICULTURE DATABASE, 2010, National Horticulture Board (ed. Bijay kumar). *Ministry of Agriculture, Gurgoan ,India*, pp.112-117

JACKSON, G.A.D., 1968. Hormonal control of fruit development seed dormancy and germination with particular reference to Rosa- Soc .*Chem. ind. (lond.) Monogr.* **30:**127-156.

JACKSON, J.E., MODLIBOWSKA, I., .SENFAZ, S.E.L., WICKENDEN, M.F., AND JOHNSON, D.S., 1982, Effect of fruit setting hormones on calcium content maturation and storage performance of Bramley's seedlings apples . *Internl. Hortl. Congress, Hamburg* **1**.

JAHNKE, S., BIER, D., ESTRUCH, J.J., AND BELTRAN, J.P., 1989, Distribution of photoassimilates in the pea plant: chronology of events in nonfertilized ovaries and effects of gibberellic acid. *Planta.*, **180**:53–60.

JIANG, Y. M., 2000, Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. *J. of Sci. of Food and Agri.*, **80**: 305–310 .

JOSHI, G.D., 1975. Studies on the spongy tissue of mango (*Mangifera indica* L.) fruits. *M.Sc. (Agri) Thesis (Unpublished). Submitted to Konkan Krishi Vidyapeeth , Dapoli (Ratnagiri), India.*

JOSHI, G.D., AND LIMAYE, V.P., 1984, Effects of manuring and harvesting time on spongy tissue occurrence in Alphonso mango. *Journal of Maharashtra Agricultural Universities.*, **9**(1): 44-46.

JOSHI, G.D., AND ROY, S.K., 1985, Spongy tissue in mango, a physiological disorder .*Indian Hort.*, **29** (4): 21-23.

KOEN, T.J., PLEISS, S.F., AND TERBLANCHE, J.H., 1990, Nutritional factors involved in physiological post harvest disorders of Avocado (CV Fuerte) . *Acta Hort.*, **182** (215): 543-550.

KATRODIA, J.S., 1979, The study into the causes of the development of spongy tissue in mango (*Magnifera indica L.*) fruit of cultivar Alphanso. *Ph.D. thesis submitted to Marathwada Agriculture University, Parbhani, India.*

KATRODIA, J.S., AND RANE, D.A., 1981, Effect of tree vigour in relation to spongy tissue development in Alphanso mango fruit. *Haryana J. Hort. Sci.* **10** (3-4): 151-154.

KALLAY, T., BUBAN T., FARAGO M., AND SZUES, F.,1987, Influence of modified source – sink relation in Jonathan apples fruits nutrition and quality . *J. Plant nutrition*, **10** (9-10): 1563-1570.

KETSA, S., 2000, Development and control of senescent spotting in Banana. *Food Preserv. Sci.*,**26**(30) :173-176.

KHODADE, M.S., 1987, Studies on physic –chemical changes during growth and development of pomegranate fruit. *M.Sc. thesis submitted to Mahatma Phule Agriculture University , Rahuri, India.*

KIRTI, S. AND NETTLES, V.F., 1961,Effect of defloration , defruiting ,nitrogen and calcium on the growth and fruiting responses of bell peppers, *Capsicum annuum* L . *Proc. Fla. State Hortic.Soc.*, **74** :204 -209.

KRISHNAMURTHY, S., 1980, Internal breakdown during ripening of Alphonso mango (*Mangifera indica* Linn.) in relation to specific gravity of the fruit . *J. Food.Sci. Technol.*, **17**: 198-207.

KRISHNAMURTHY, S., 1981, Chemical studies on internal breakdown in Alphanso mango (*Magnifera indica L.*).*J. Hort. Sci.*, **56**(3): 247-50.

KULKARNI, A. P. AND ARADHYA, S. M., 2005, chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chem.*, **93** (2): 319-324.

KUMAR, G.N.M., 1990, Pomegranate. In: Fruits of tropical and subtropical origin. Nagy, Show and Werdowski (eds). *Florida Sci. Source, Inc., Florida*, USA, pp. 328-347.

LARRIGUADIERE, C., PINTO, E., LENTHERIC, I. AND VENDRELL, M., 2001, Involvement of oxidative processes in the development of core browning in controlled –atmosphere stored pears; *J. Hortl Sci Biotechnol* ., **76**: 157-162.

LAU, O. L., 1998, Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. *Postharvest Biol.Technol.*,**14**: 131-141.

LIMAYE, V.P., JOSHI, G.D. AND GUNJATE, R.T., 1976, Occurence of spongy tissue in relation to the stage of harvest of Alphonso mango fruits. *J.Maharashtra Agril. Univ.*, **1** :96 -104.

LIDSTER, P.D., PORRITT, S.W., EATON, G.W. AND MASON, J.,1975, Spartan apples breakdown as affected by orchard factors , nutrients content and fruit quality .*Can. J. Plant Sci.* **55**: 443-446.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. AND RANDALL, R.J.,1951, Protein measurement with Folin Ciocalteau Phenol Reagent. *J. Biol. Chem.*, **193**: 265-275.

MAEPRASART, D., VEARASILP, S., AND KRITTIGMAS, N., 1999, Dehydrogenase enzyme level and seed qualities in green soybean seeds produced under different planting dates. *Duetscher Tropentag* , Berlin.

MAGINESS, J.R., DIEHL, H.C., AND ALLEN, F.W., 1929, Investigations on the handling of Barlett pears from Pacific Coast .*USDA Tech Bull.* **140** , pp 28.

MALICK, C.P. AND SINGH, M.B., 1980, In: Plant Enzymology and Histoenzymology . *Kalyani Publications, New Delhi*, pp 286.

MARCELIS, L.F.M. AND HEUVELINK, E., 1990, Effect of assimilate supply on fruit growth in cucumber and tomato. *Abstracts of contributed papers, 23rd Internal Horticulture congress, 27Aug- 1 Sept 1990, Florence, Abstr, 3406.*

MARTIN, D., 1954, Variations between apple fruits and its relation to keeping quality III . *Australian Jour. Agr. Res*. **5** : 392 -421.

MOTA, V. R., SOUZA, R C., SILVA, C. P. C., FREITAS, F. G., SHIGA, M. T., PURGATTO, E., LAJALO, M. F. AND REGINA, A. M., 2010, Biochemical and agronomical responses of grapevines to alteration of source-sink ratio by cluster thinning and shoot trimming, *Bragantia.*, **69:** 1-3.

MURNEEK, A. E., 1926, Effect of correlation between vegetative and reproductive functions in the tomato (*Lycopersicon enculentum* Mill.) *Plant Physiology*, *Lancaster* **1** :53-55.

NATH, V., KUMAR, D. AND PANDEY, V., 2008, Fruits for the future (Vol.1 : Well versed arid and semi arid fruits). *Satish serial publishing house*, pp. 103-127.

NAUMANN, G., 1974, Second discussion meeting on bitter pit in apples. *Acta Hortic*, **45** .

NITSCH, J.P., 1970, Hormonal factors in growth and development. In: Hulme AC, editor. *The biochemistry of fruits and their products. London and New York: Academic Press*, p 427–472.

NOCTOR, G. AND FOYER, C., 1998.Ascorbate and glutathione : keeping active oxygen under control . *Ann. Rev Plant Physiol. Plant Mol.Biol.* **49** : 249-279.

NYHLEN, A., AND ROOTSI, N., 1961, Nagra ron om pricksjuka. Sver.Pomol. Foren, Arsskr, *Hort. Abstr.*, **31**: 4039.

OPARA, L.U., STUDMAN, C.J., AND BANKS, N.H., 1997, Fruit skin splitting and cracking. *Hortic Rev.* , **1**: 217-262.

PAULL, R.E. AND REYES, M.E.Q., 1996, Preharvest weather condition and pineapple fruit translucency .*Sci Hortic.*, **66**: 59-67.

PATKAR, V.R., 1978, Studies on chemical composition during growth and development and effect of growth regulators on ripening of Alphanso mango fruits with particular reference to spongy tissue. *M.Sc.(Agri)Thesis(Unpublished).Submitted to Konkan Krishi Vidyapeeth , Dapoli (Ratnagiri),* India.

PAWAR, S.K., DESAI, O.T. AND CHOUDHARI, S.M., 1994, Effect of pruning on physiological disoders in pomegranates. *Ann Arid Zone.,* **33**: 83-84 .

PHARIS, R.P. AND KING, R.W., 1985, Gibberellins and reproductive development in seed plants. *Annu Rev Plant Physiol.,* **36**:517–568.

PERKINS, D.Y., MILLER, J.C. AND DALLYN, S.L., 1952.Influence of pod maturity on the vegetative and reproductive behaviour of okra. *Proc.Am.Soc.Hortic Sci.*, **50**: 311-314.

POWELL, A. A. AND KREZDROM, A.H., 1977, Influence of fruit –setting treatment on translocation of ^{14}C – metabolites in citrus during flowering and fruiting. *J. Am.Hortic Sci.*, **102**:709-714.

RANGWALA, A.D., 1975, Changes in chemical composition of alphanso fruits during ripening with particular reference to spongy tissue . *M.Sc.(Agri) Thesis (Unpublished).Submitted to Konkan Krishi Vidyapeeth , Dapoli (Ratnagiri), India.*

RAPHAEL, J. AND O'LOUGHLIN, J.B.,1964, Maturity studies on Cox'sOrange Pippin. *Tasamanian Jour.Arg.*, **35**: 56-58.

RAVINDRA, V. AND SHIVASHANKAR, S., 2004, Spongy tissue in Alphaonso mango significance of in situ seed germination events, *Curr. Sci.*, **87**:1045-1049.

RICHARD, F.C., AND GAUILLARD, F.A., 1997, Oxidation of chlorogenic acid, catechins and 4-methylcatechol in model solution by combinations of pear (*Pyrus communis cv.Williams*) polyphenol oxidase and peroxidase : a possible involvement of peroxidase in enzymatic browning . *J.Agric Food Chem* ., **45** : 2472-2476.

ROSA, J.T., 1924, Fruiting habit and pollination of cantaloupes. *Proc.Am. Soc. Hortic.Sci.,* **21**: 51-57.

SADASIVAM, S. AND THEYMOLI, B., 1987, In: *Practical Manual in Biochemistry*. Tamil Nadu Agriculture University Coimbatore , pp 14.

SALISBURRY, B.F. AND ROSS, W.C., 1986, *Plant physiology (Third Edition), Word Sworth Publishing Company , USA,* pp 309 – 349.

SAWANT, B. A., 1993, Biochemical investigation of pomegranate (*Punica granatum* L.) arils in relation to blackening. *M.Sc.(Agri) thesis submitted to MPKV,Rahuri ,India.*

SCHUMACHER, R., 1978, Physiological disease of apples –cause, prevention and treatment. *Bassers obst.*, **26**(6): 93-95.

SCHUMACHER, R., FANKHAUSER, F., MULLER, V. AND STADLER, W.,1981, Influence of calcium chloride treatment on the bitter pit incidence and Ca, K and Mg content of fruit flesh and fruit juice. *Schwiezerische Zeitschrift fiir obst. Und Weinban.*, **117** (27): 783-789.

SEKSE, L., 1995, Fruit cracking in sweet cherries (*Prunus avium* L.). Some physiological aspects – a mini review, *Sci Hortic.*, **63** : 135-141.

SELVARAJ, Y., RAJA, M.E. AND RAWAL, R.D., 2000, Biochemical studies on Internal Breakdown – A ripening disorder in Mango fruits. *Indian J. Hort.*, **57** (3) 183-188.

SHARMA, R.R. AND SINGH, R., 2009, The fruit pitting disorder—A physiological anomaly in mango (*Mangifera indica* L.) due to deficiency of calcium and boron. *Sci. Hort.*, **119** (4) :388-391.

SHETE, M.B., 1998, Effects of varieties, stage of harvest and bahar treatment on internal breakdown of pomegranate (*Punica granatum* L.) fruits .*Ph.D thesis submitted to mahatma Phule Agriculture University, Rahuri, India.*

SHETE, B. AND WASKAR, D. P., 2005, Internal breakdown of Pomegranate (*Punica granatum* L.) fruits – a review. *J. Maharashtra Agric. Univ.,* **30** (10):59-61.

SHIVASHANKAR, K. S., SUBHAS, C. M., LAXAM, R.H., VIJAYALAXMI, G.P. AND BUJJIBABU, C.S., 2004, Physiological and biochemical changes associated with aril browning of pomegranate (*Punica granatum* cv. Ganesh). *J. Plant Biol.*, **31** (2) :149-152 .

SHIVASHANKAR, S., RAVINDRA, V. AND LOUIS, L., 2007, Biochemical changes in seed and mesocarp of mango (*Mangifera indica* L.) cv. 'Alphonso' and their significance during the development spongy tissue. *J. Hort. Sci. Biotech.*, **82** (1): 35-40.

SMITH, 1926, Bitter pit in apples, a review of the problem. *Food Inv.Board (Great Britain) Spec.Rep*. **28**, 23 pp.

SMOCK, R.M., 1941, Studies on bitter pit in apple. *Cornell. Univ. Agric. Expt. Station Memoir.*, **234**:45.

SOLER, A., 1994, Enzymatic characterization of stress induced translucence of pineapple flesh in the Ivory Coast. *Acta Hortic.*, **334**: 295-304.

STIGTER, H.C.M., 1969, Growth relations between individual fruits and between fruits and roots in cucumber. *Netherlands Journal of Agric Sci.*, **17**: 209 -214.

SUBRAMANYAM, H., KRISHNAMURTHY S., SUBHADRA, N.V., DALAL, V. B., RANDHAWA, G.S. AND CHACKO, E.K., 1971, Studies on internal breakdown, a physiological ripening disorder in alphanso mango (*Mangifera indica* L.).*Trop. Sci.*, **13** (3):203-210.

SUNG, F.J.M. AND CHEN, J.J., 1988, Tetrazolium test for predicting the seedling vigor of rice at optimal and temperatures. *Crop Sci.,* **28**(6):1012- 1014.

SUNDARRAJ, R.M., SINGH, H.D. AND SURESH, E.R., 1972, Methods of statistical analysis. *J.App.Stat.*, **14** : 124-128.

SZYNKLER, K., 1974, The effect of removing of supply or sink organs on the distribution of assimilates in two varieties of garden pea, *Pisum sativum* L. *Acta Agric. Scand.*, **24**:7-12.

TOBAR, S.M., TEHRANIFAR, A., DAVARYNEJAD, G.H., NEMATI, S. H. AND ZABIHI, H.R., 2009, Aril Paleness, new physiological disorder in pomegranate fruit (*Punica granatum)*: Physical and chemical changes during exposure of fruit disorder. *Hort Environ. Biotechnol.*, **50**(4):300-307.

TUSTIN, D.S., WATKINS, C.B., CASHMORE, W.M. AND BROOKFIELD, P.L., 1993, Source of variation in fruit quality of 'Braeburn' apple at harvest and storage. *HortSci.*, **28**: 446(abstract).

UNDERHILL, S.J.R., CRITCHLEY, C., SIMONS, D.H., AND SUBHADRABANDHU S., 1992, Postharvest pericarp browning of lychee (*Litchi chinensis Sonn.*); *Acta hortic* .,**321**:718-725.

USDA NATIONAL NUTRIENT DATABASE, 2010, *United States Department of Agriculture*, Release 23.

VARGA, A. AND BRUINSMA, J., 1976, Roles of seeds and auxins in tomato fruit growth. *Zeitschrift fu*xrp *anzenphysiologie.,***80**: 95-104.

VAN GOOR, B.J., 1971, The effect of frequent spraying with calcium nitrate solution on the mineral composition and the occurrence of bitter pit of the apple Cox's Orange pippin, *J.Hort. Sci.*, **46**:347-364.

VELTMAN, R.H., SANDERS, M.G., PERSIJIN, S.T., PEPPELENBOS, H.W. AND OOSTERHAVEN, J., 1999, Decreased ascorbic acid levels and brown core development in pears (*Pyrus Communis* cv 'Conference'). *Physiol.Plant.*, **107** :39-45.

WALKER, A.J. AND HO, L.C., 1977, Carbon translocation in the tomato: Carbon import and fruit growth. *Ann.Bot.*, **41**:813-824.

WASKER, D.P., KULKARNI, S.P., NAVALE, A.M. AND SHETE, M.B., 2003, 'Bhagwa' and 'Phule Arka'. *Mahatma Phule krishi Vidyapeeth , Extension. Bull. No*. **410**.

WHETZEL, H.H., 1912, Baldwin spot or stippen. *New York State Fruit Grow.Ass.Proc.*, **11**: 28-34.

WITNEY, G.W., KUSHAD, M.M., AND BARDEN, J.A., 1991, Induction of bitter pit in apple. *Scientia Hortic.* **47**(1-2):173-176.

XINGHUA, L., QINXIA, H., LIPING, K., AND SONGSHEGU, O., 1988, Biochemical characteristics studies on peel browning of pomegranate after picking ; *J. North West For College.* **13**; 12-19.

YUJI, I., SATORU, O., HIDEAKI, Y. AND KUNIHIKO, S., 1996, Effect of gibberellins and their synthesis inhibitors on the occurrence of watercore in 'Housi' Japanese pear. *Bull. Fruit Tree Res. Stn.*, **29** : 51-65.

ZAUBERMAN, G.R., ROREN, R.,AKERMNA,M., WEKSLER, A., ROT, I., AND FUCHS, Y., 1991,Postharvest retention of the Red colour of litchi fruit pericarp. *Scientia Hortic* .**47** : 89-97.

ZERBINI, P.E., RIZZOLO, A., BRAMBILLA , A., AND GRASSI , M., 2002, Loss of ascorbic acid during storage of Conference pears in relation to the appearance of brown heart. *J. Sci. Food Agric.*, **82**:1-7.

ZHANG, Z.Q., PANG, X.Q., JI, Z.L. AND JIANG, Y.M., 2001, Role of anthocyanin degradation in litchi pericarp browning.*Food Chem.*, **75**: 217-221.

ZHANG, Z.Q., PANG, X.Q., JI, Z.L. AND JIANG, Y.M., 2005, Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chem.* **90**:41-52.

ZHONHONJI, T., LIANGNAN, T., JIASHENG, Y. AND LIN, Y., 1981, The occurrence and control of water core cv. Delicious apples in Maqin Ling Region. *Acta Hor.,* **8**(4):1-8.