School of Science

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Influence of Infections of Mild Isolates of Different Grapevine Viruses on Berry Colour, Texture, Flavour and Storage Life of 'Crimson Seedless' Table Grapes

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: _____

Date: _____

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Abstract

‘Crimson Seedless’ grape is an economically important cultivar of table grapes with superior eating characteristics due to firmer berries, colour and good flavour. The aim of this research project was to investigate the influence of infection of mild isolates of grapevine leafroll associated viruses (GLRaV) 3, 5, 9 and grapevine virus A (GVA) on berry colour, texture, SSC, TA and SSC: acid ratio in ‘Crimson Seedless’ grapes during maturation, ripening and cold storage life and quality. The infection of GLRaV and GVA viruses in clone 3215 (LRV3 (E) + LRV3 (RT-PCR) + GVA + LRV9 + LRV5), clone 3236 + 3215 (LRV3 (E) + GVA + LRV9 + LRV5) reduced berry colour, SSC, SSC: acid ratio; improved berry springiness and gumminess without influencing acidity during maturation and ripening in comparison to virus free control. During cold storage, berries from viral infected clones 3236 + 3215 and 3215 showed improvement in berry colour and SSC and retained good quality until 140 days of storage but, there was no effect on acidity. Berry hardness, gumminess, springiness and cohesiveness were also higher in viral infected clone 3236 + 3215 and clone 3215 than virus free control during cold storage. In sensory evaluation, virus infected clones 3236 + 3215 and 3215 obtained higher scores for berry crispiness, flavour and overall acceptability during cold storage when compared to the virus free controls. In conclusion, the infection of mild isolates of GLRaV and GVA viruses reduced berry colour and SSC but, improved berry textural properties in clones 3236 + 3215 and 3215 of ‘Crimson Seedless’ grapes during maturation and ripening. The quality parameters such as berry colour, textural properties, SSC, TA and sensory scores also remained acceptable for these clones till 140 days cold storage at 0 ± 0.5^{o}C.

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$	Dollar
%	Per cent
/	Divide
^{o}	Degree
^{o}F	Fahrenheit
±	Plus minus
×	Multiply / interaction between
≤	Less than or equal to
^{o}C	Degree celsius
ABA	S-(+)-cis, trans-Abscisic acid
ABS	Australia Bureau of Statistics
ANOVA	Analysis of variance
ATGA	Australian table grape association
ATGI	Australian table grape industry
BW	berry weight
C*	Chroma
CA	Controlled atmosphere
Cn3glc	Cyanidine 3-*O*- glucoside
CO_2	Carbon dioxide
Cv	Cultivar
DAFWA	Department of Food and Agriculture Western Australia
DAV	days after veraison
Dp3glc	Delphinidin 3-*O*-glucoside
ds RNA	double stranded RNA
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agriculture Organisation
Fig.	Figure
g	Gram
GA_3	Gibberellic acid
GFLV	Grapevine fanleaf virus
GFKV	Grapevine fleck virus
GLRaV	Grapevine leafroll associated virus (s)

GVA	Grapevine virus A
GVB	Grapevine virus B
h	Hour
h^o	Hue angle
ha	Hectare
HDPE	High density polyethylene
Kg	Kilograms
L	Litre
L*	Lightness
LSD	Least significant different
Ltd	Limited
m	Metre
M	Molar
MAP	Modified atmospheric package
mg	Milligram (s)
mL	Millilitre
mm	Millimetre (s)
mm s^{-1}	Millimetre per second (s)
mol/kg	Moles per kilogram
MT	Million tonne (s)
Mv3glc	Malvidin 3-*O*-glucoside
N	Newton
NaOH	Sodium hydroxide
N-OPP	Non-perforated polypropylene
ns	not significant
nt	nucleotide
O_2	Oxygen
PCR	Polymerase chain reaction
pH	Symbol denoting hydrogen ion in a solution
Pn3glc	Peonidin 3-*O*-glucoside
RH	Relative humidity
RNA	Ribonucleic acid
RSPaV	Rupestris stem pitting
RT-PCR	Reverse transcriptase polymerase chain reaction

RW	Rugose wood
s	Second (s)
sp	Species
SSC	Soluble solids concentration
TA	Titrable acidity
TPA	Texture profile analyser
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
WA	Western Australia
µg	Microgram (s)
µL	Microliter (s)

CHAPTER 1
General introduction

Grapevine is the world's most widely grown fruit plant of all the horticultural crops with a cultivated area of 7, 437, 141 ha and production of 66, 935, 199 MT (FAOSTAT, 2009). Australia's table grape industry ranks first among all the other horticultural industries (ATGA, 2010). Australian grape industry produces 140, 000 MT grapes annually from an area of 10, 500 ha (ATGI, 2009). About 50% of this production is exported to world markets with an export value of $190 Million. Table grapes are produced in all the states of Australia including Victoria, New South Wales, Queensland, Western Australia (WA) and South Australia but, South Australia, Victoria and New South Wales are the largest grape producing states. WA contributes 86, 421 MT to the annual total grape production in Australia (ABS, 2009). WA produces table grapes of international quality and thus fetches premium prices in both domestic and international markets (Cameron and Pasqual, 2004).

The demand for Australian grapes gained impetus in the world markets for the past decades. Australian environmental conditions are suitable for the production of world class table grapes and the land mass from tropics to temperate in south west allows production of fresh table grapes from November to May (ATGI, 2009). Further, cool storage extends the supply of fresh table grapes throughout the year. The most economically important table grape cultivars grown in Australia are 'Flame Seedless', 'Dawn Seedless', 'Menindee Seedless', 'Red globe', 'Crimson Seedless', 'Thompson Seedless' (Hannah and Pitt, 2004). 'Crimson seedless' is one among the most dominating red skinned cultivars in Australia. It is a cross between 'Emperor' and 'C 33-199' and was released for its cultivation in WA during 1996 (Cameron, 2001) and performed well in all regions of WA from Gingin to Donnybrook. 'Crimson Seedless' berries are elongated, crisp, firm and have sweet neutral juicy flavour. It is a late season variety matures from late February to late March. 'Crimson Seedless' cultivar is becoming an important commercial cultivar in table grape industry of WA. The most important criteria for good quality grapes are firm texture, attractive colour, rich flavour and overall acceptability (Cameron, 2007; Sato and Yamada, 2003). Various physiological and physical changes occurs in berry during ripening such as decrease in acids, accumulation of sugar, and anthocyanins, changes

in flavonols, increase in solute content, softening of berries (Coombe and Bishop, 1980). Berry ripening process strongly influenced by many factors such as environment, water relations, cultural practices, viral infection, and cultivar (Mullins et al., 1992).

The effect of grapevine viruses on the quality of table grapes has been studied in WA over 30 years (Brar et al., 2008). There are nine serologically proved leaf roll viruses which can cause infection in grapevines. Amongst these isolates, grapevine leafroll associated viruses (GLRaV-3 and GLRaV-1) are considered to be the most virulent in causing deleterious effects to both vine growth as well as quality of grape berry. Infection of these leafroll viruses leads to low sugar accumulation, decrease in anthocyanin content and delayed maturity (Cabaleiro et al., 1999; Guidoni et al., 1997). However, the infection of mild isolates of these viruses exhibits positive effects on table grapes such larger berry size. 'Crimson Seedless' clones have been identified with infection of leaf roll viruses 3, 5, 9 and Grapevine associated virus in WA, which produces 20-25% bigger berries than the world standard (Brar et al., 2008). Texture and colour are the main quality parameters which influence the consumer's acceptability (Jayasena and Cameron, 2008). 'Sultana' clones with leafroll infection were reported to produce larger berries. Mild infection of leafroll virus in clones of 'Emperor' showed better performance with crispness of berries than the virus free clones (Jayasena and Cameron, 2008). Limited research work has been reported on the effects of infection of mild isolates of GLRaV, grapevine virus A (GVA) on ripening process and quality parameters. The effects of these isolates on changes in the textural properties of grape berry during ripening warrants to be investigated.

Various post-harvest approaches have been tested to minimise the losses in grape berry quality parameters such as appearance, texture, flavour, nutritive value to reduce post-harvest losses (Zutkhi et al., 2001). Post-harvest losses in perishable horticultural commodities have been estimated 5 to 25% in developed countries and 20 to 50% in developing countries (Kader, 2002). Consumer tracking study of table grapes shows that more than 70% of consumers lack confidence on the product quality of table grapes (ATGA, 2010).

Table grapes exhibit very low respiration rate and can be stored for a long time with postharvest measures. Grapes are subjected to major losses during storage period such as water loss, berry decay, storage pest and fungal pathogens (Crisosto et al., 2001; Deng et al., 2006). During the past 50 years, usage of controlled atmosphere (CA) and modified atmospheric packages (MAP) has been increased in the area of post-harvest technology of fruit to maintain the quality of the produce. 'Flame Seedless' with N-OPP film (MAP) remained good in quality even after 53 days of storage at 1^{o}C with better firmness, without off flavours and maintained better colour than control (Martinez-Romero et al., 2003). SO_2 pads were used commercially to minimise the infection of fungal pathogens in postharvest storage practices. A SO_2 pad with a concentration of 100 ppm per hour was considered as the most effective in control of fungal infection (Zoffoli et al., 1999).

Infection with mild strain of learoll virus showed 15% increase in berry weight in 182 'Sultana' grape clones over virus free clones (Antcliff et al., 1979). The virus infected clones 314 and 306 with a combination of viruses such as GVA, GLRaV-9, GLRaV-3 and Rupestries stem pitting virus (RSPaV), introduced from WA research, has been proved to produce large and crisper berries when compared to the virus free standard clones and they maintained the quality even after one month of cold storage (Jayasena and Cameron, 2008).

As a prelude, no research work has been reported on the influence of infection of mild isolates of grapevine leafroll virus infection on textural property of 'Crimson Seedless' berries during maturation and ripening. Further, the effects of grapevine leafroll viral infection on the storage life and quality of 'Crimson Seedless' grapes particularly the textural properties of berry during long cold storage have also not been investigated.

Objectives:

1. The main focus of this research was to elucidate the effects of infection of mild isolates of GLRaV, GVA on the textural properties and other quality parameters of 'Crimson Seedless' berry during maturation and ripening.

2. To uncover the influence of mild isolates of GLRaV, GVA infection on the quality, textural properties and storage life of ‘Crimson Seedless’ grapes during cold storage.

CHAPTER 2

General literature review

2.1. Grapes

2.1.1. Origin of grapes

Grapes originated in Southern Caucasia now known as north West-Turkey and Northern Iraq. It was introduced into Australia in 1788. Grapes belong to family *Vitaceae*, and there are about 12 genera classified in this family. Genus *Vitis* is the most cultivated in world's grape growing regions. *Vitis Vinifera* L. occupies 90% of the total approximately 10,000 grape cultivars in the world (Winkler et al., 1974). Grapes are used in different forms such as table grapes, raisin, wine grapes, sweet juice and canning grapes (Winkler et al., 1974).

2.1.2. Grape production

Grapes are one of the most important fruit crops in the world which ranks first in growing area (7, 437, 141 ha) and second in production (66,935,199 tonnes) next to Banana (FAOSTAT, 2009). Northern hemisphere produces 85% of the world's total table grapes with Italy, China, USA, Spain, Turkey, Greece and Mexico as the largest producers accounting 9 million tonnes (MT) of table grapes (Figure 2.1). In southern hemisphere Chile, South Africa and Australia produces about 1.4 MT of table grapes annually (ATGI, 2009).

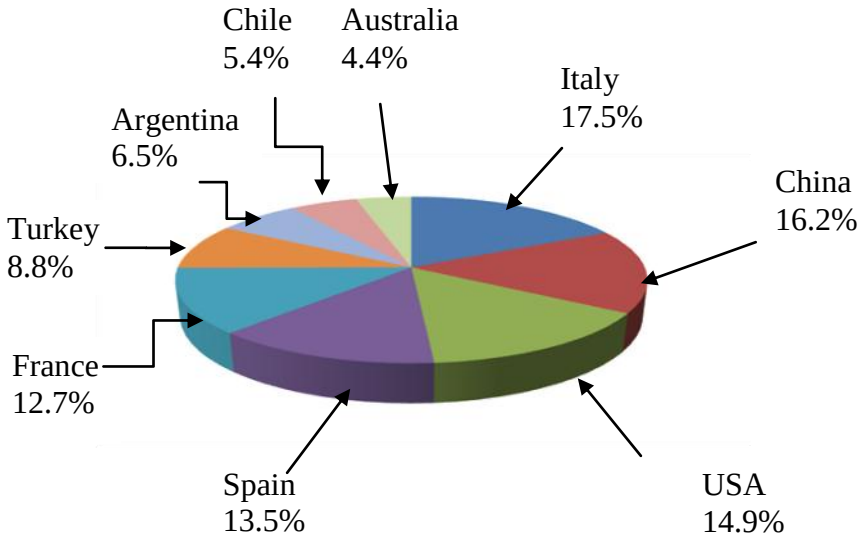

Figure 2.1 World production of grapes (FAOSTAT, 2009)

2.1.3. Australian grapes market

Australia ranks 9th in the world for grape production, produces about 1,956,790 tonnes of grapes from an area of 10,500 ha (FAOSTAT, 2009). Australia contributes around 2% of the total table grape exports in the world market. Australian table grape industry is one of the country's fastest growing horticultural industries with an increase in production from 30,000 to 1,956,790 tonnes during the past decades. South Australia has got the largest area under vineyards with 46.5% of total national vineyards area, followed by New South Wales (25.8%) and Victoria (18.5%) (ABS, 2009). There are large number of table grapes varieties grown in Australia but, 'Flame Seedless', 'Dawn Seedless', 'Menindee seedless', 'Thompson Seedless', 'Crimson Seedless' and 'Red Globe' are becoming more popular among the grape growers (ATGI, 2009).

2.1.4. Stages of grape berry growth

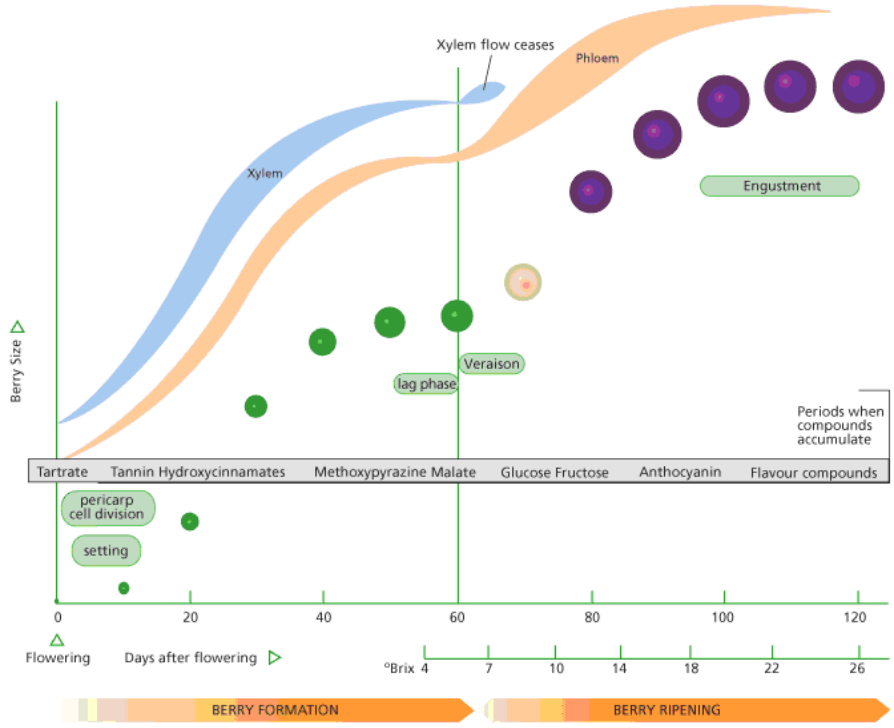

Figure 2.2 Grape berry growth, development and ripening (Kennedy, 2002)

Botanically, the grape fruit is termed as 'berry' which contains seed inside the pulp (Creasy and Creasy, 2008). Growth of the berry begins immediately after the completion of anthesis. Berry mass increases about 4,000 fold starting from anthesis to ripeness (Coombe, 1976). Pericarp consists of three different tissues anatomically.

exocarp (skin), the mesocarp (pulp) and the endocarp inner most part of the pericarp (Mullins et al., 1992). The volume of pericarp bounds to increase by volume 10-20% during anthesis and extends to 65% at maturity (Mullins et al., 1992). The grape berry growth follows a double sigmoid growth pattern (Iwahori et al., 1968) having three distinct stages as shown in (Figure 2.2) and discussed as under:

2.1.5. Stage I

Berry formation and development of embryo takes place in this first stage of the growth phase. Rapid cell division and cell enlargement is the most prominent aspect of this phase for the first few weeks and during the end of this stage entire number of cells are formed (Harris et al., 1971). Cell division starts its cessation from placenta to inner pericarp (Considine and Knox, 1979). Accumulation of several solutes and expansion of berries will take place in this period (Possner and Kliewer, 1985). Berries remain greener, harder and accumulation of organic acids is noted in this stage. The most predominantly occurring acids at this phase are tartaric and malic acid which constitute major composition of titrable acidity (TA).

2.1.6. Stage II:

This phase continues for 7-40 days and is generally described as a lag phase or slow growth phase. During this stage loss of chlorophyll occurs resulting into decreased rate of photosynthesis occurs and seeds attain their maturation (Mullins et al., 1992; Winkler et al., 1974). Whereas, berries start to soften and acidity will be reaching their maximum point (Coombe and Hale, 1973). This phase will be extended in late maturing grape cultivars (Coombe and Bishop, 1980). It is influenced by cultivar, environment, cluster appearance, and flowering time (Coombe and Hale, 1973; Nakagawa and Nanjo, 1965; Pratt, 1971).

2.1.7. Stage III:

Stage III lasts for 5-8 weeks and during this period berry softens and anthocyanins accumulate in coloured cultivars. Decrease in TA and accumulation of hexose sugars reaches to maximum during the end of this stage (Mullins et al., 1992; Winkler et al., 1974). No more cell division takes place in this stage (Coombe, 1960). In some of the grape cultivars loss of the berry weight was noted during harvesting time (McCarthy, 1999).

2.2. Changes during ripening

Grapes are non-climacteric fruit (the fruit that does not ripen after detachment from the plant) but, the ripening is very distinct in grape berries. Many physiological changes occurs in this ripening period most of them are more prominent needs only less time to change about 24-48 hours (Mullins et al., 1992). There is a marked change in levels of organic acids such as decrease in tartaric acid, malic acid and a sudden increase in sucrose, fructose and glucose levels. Berry softening, increase in the deformability, decrease in chlorophyll synthesis and accumulation of anthocyanins (Coombe, 1992; Coombe and Bishop, 1980; Coombe and Hale, 1973). The massive increase in sugars with decrease in organic acids indicate a shift in translocation pattern (Mullins et al., 1992).

Ethylene is a plant hormone which remains low throughout the berry development (Coombe and Hale, 1973) but the exogenous application of ethylene in the form of ethrel® hasten the fruit ripening (Coombe and Hale, 1973; Hale et al., 1970). Gibberllins and cytokinins are found to be more pronounced in the early growth phase later on it seems to decline during ripening phase. Auxin and absicic acid (ABA) concentrations decrease till veraison, whereas during ripening, there was an increase in ABA levels and decrease in auxin levels (Cawthon and Morris, 1982; Coombe and Hale, 1973; Inaba et al., 1976; Scienza et al., 1978).

Phenolic compounds occur in the plant tissues naturally (Wilson and Allen, 1994). These compounds play an important role in the quality of grapes by influencing colour, flavour, and taste. They are sub-grouped as flavonoids and non-flavonoids (Montealegre et al., 2006). Flavonoids are divided as anthocyanins and flavonals (Downey et al., 2006). In grapes these total phenols decreases until veraison and starts increasing during ripening in coloured varieties (Kataoka et al., 1983).

2.2.1. Berry softening

Berry firmness is one of the main criteria in the quality of grapes as firmness is often compared with freshness and crispness (Bernstein and Lustig, 1985). Berry crispness is one of the most desirable factor for the table grapes (Sato and Yamada, 2003). A good quality grapes should have firm texture and good flavour (Hannah and Pitt, 2004). Softening is a process of ripening of the berry and it commences at the stage II of berry development (described above in section 2.1.6.). Three mechanisms are generally thought to be involved in the softening of fruit. Loss of turgor pressure,

degradation of starch, break down of cell walls (Seymour et al., 1993). Berry skin cells of the grapes mainly depend upon cultivar. They contain cuticle, lenticels, wax and collenchymatous hypodermal cells (Winkler et al., 1974).. Around 50% of the composition of cell wall constitutes of pectin substances (Jona and Foa, 1979). The softening enzymes such as pectin methylesterase and polygalacturonase are responsible for decreasing the pectin content during ripening process. Berry skin thickness is related to the sugar content in the pulp dependent upon the area of cultivation (Torchio et al., 2009). Grape maturity is one of the main criteria in wine making process, texture analysis is the best method for sorting the maturity, since they are able to find out the appropriate time of ripening of phenolics (Rolle et al., 2007). Anthocyanins extractability depends on hardness of the skin (Rolle et al., 2009). This texture profile analysis can be used as varietal difference markers (Río et al., 2008). The first method developed to measure the textural properties was done by Morris (1925). Terminologies used in textural profile analysis are hardness, cohesiveness, elasticity, brittleness chewiness and gumminess.

2.2.1.1. Hardness: Hardness is also termed as firmness (Henry et al., 1971; Sheerman, 1969). Mechanically it is a peak force that appears in the first compression cycle (Table 3.2).

2.2.1.2. Springiness: Springiness is substituted term for elasticity (Massey and Woodham, 1973). It is explained as height of the food recovery during the elapsing time between the first bite and the start of the second bite.

2.2.1.3. Adhesiveness: Adhesiveness is referred as the negative area of the first bite (Bourne et al., 1974), and the work needed to pull the probe from sample.

2.2.1.4. Cohesiveness: The term cohesiveness is referred for cohesion (Saxton and Jewell, 1969). The ratio of the work during compression of the second cycle divided by that of the first cycle.

2.2.1.5. Brittleness: Brittleness was defined as bio yield point (Bourne et al., 1974), defined as fracturability the term for break. The first significant break made by the first compression cycle.

2.2.1.6. Chewiness and gumminess: Chewiness and gumminess is known as product of Hardness × Cohesiveness × Springiness (Bourne, 1978).

2.2.2. Colour

Colour is one of the most important criteria in defining the quality of grapes. Generally colour change is referred as loss of chlorophyll and *de novo* biosynthesis of certain pigments (Darby et al., 1977). Veraison is the term generally used to define growth phase linked to colour change during initiation of ripening. Pigments that involve in colouration of red coloured variety are anthocyanins. According to the skin colour, the grape berries are classified as white, red, black varieties (Kanellis and Roubelakis, 1993). The accumulation of anthocyanins in pigmented cultivars starts predominantly at veraison and continues to accumulate during berry maturation and ripening. They are found to be present in the vacuoles of 1-3 subepidermal layers of grape berry skin which are below the epidermis, whereas some of the varieties may differ in the position of their presence as they may be found in mesocarp cells (Mullins et al., 1992). 3-*O*-monoglucosides of delphidine, cyanidine, petudine, peonidine and malvidine are the main anthocyanin constituents of Vinifera grapes and these compound are reported to be found along with their acyl derivatives (Winkler et al., 1974; Wulf and Nagel, 1978). The amount of anthocyanin concentration present in the grapes ranges from 30-750 mg/100 g in fresh ripened berries (Mazza, 1995). The skin of black grapes are richest source of anthocyanins than other varieties (Timberlake, 1980). Various factors such as species, cultivar, temperature, light, crop load influenced the composition and amount of anthocyanin content in grape berries (Mazza, 1995).

2.2.3. Soluble solids concentration (SSC) and titrable acidity (TA)

Berries undergo remarkable changes during the process of maturation and ripening. Maturation of grapes includes the changes in both physical and chemical properties. Maturation starts at the veraison stage and lasts for 40-50 days until the berry is fully ripe. Grape berries are found to contain sugars such as glucose, fructose, sucrose, maltose, galactose, melibiose, raffinose, stachyose (Kliewer, 1965). The accumulation of sugars during ripening period is coincided with berry softening (Coombe, 1989; Kanellis and Roubelakis, 1993). Sucrose produced as a result of photosynthesis is further hydrolysed by the enzyme invertase into glucose and fructose which constitutes 99% of the SSC (Hardy, 1968; Kliewer, 1966; Peynaud and Maurie, 1958). Whereas, glucose is the major predominant sugar during veraison (Winkler et al., 1974). As sugar increases, the organic acids are bound to decrease

during ripening. The most dominant part of acids was constituted by tartaric and malic acids which represent about 90% of total TA. Grapes are well known for its substantial amount of tartaric acid among other fleshy fruits (Mullins et al., 1992; Ruffner, 1982). Catabolism of tartarate molecules occurs at slower rate throughout the berry development. A considerable decrease in the concentrations of both malic acid and tartaric acid was noted until veraison but after veraison decrease in malic acid content is more tremendous (Hardy, 1968; Iland and Coombe, 1988). Whereas, the decrease in tartaric acid content is found to be at lower rate or often its concentration remains constant after veraison, which can be traced on per berry basis (Iland and Coombe, 1988; Kliewer, 1964). Hence, the decrease in TA is mainly due to the decrease in malate contents (Mullins et al., 1992). Tartaric acid is the most predominant acid found in berries at the end of maturation (Kliewer, 1964; Kliewer et al., 1967). These phenomenon were being well exhibited in cultivars such as 'de Chaunac' (Hrazdina et al., 1984), 'Monastrell', 'Cabernet Sauvignon', 'Tempranillo' (Gómez and Martinez, 1995). Harvesting index was given according to the acid level of the fruit such as low acid varieties must be harvested with SSC: acid (TA) as maturity indices. Grapes with high acid content to be harvested with consideration of acid content, whereas grapes with medium acid content must be harvested considering both SSC and TA (Coombe and Bishop, 1980).

2.2.4. SSC: acid ratio

SSC: acid ratio prevails to be the best maturity index than individual values of sugars and acids (Coombe and Bishop, 1980). Since maturity is the main criteria in the wine making process, SSC: acid ratio can be used as reliable markers for identifying maturity (Jayasena, 2008). There will be a steady increase in the level of SSC: acid ratio along with that of SSC as the ripening pronounces (Al-Kaisy et al., 1981; Flora and Lane, 1979).

2.3. Grapevine virus infection

Among virus and virus like diseases, grapevine viruses have been reported to cause deleterious effects on grapevine cultivation over 100 years (Goheen and Cook, 1959). Approximately, there are about 58 species of viruses found to infect grapevines (Martelli, 1993). They were called by different names in different countries for many reasons. In France, the symptoms were called as Rougeau,

Flavesence and Rollkrankheit; in Germany it was named as red leaf symptom (Over de Linden, 1970). At first it was described as potassium deficiency later on the work done by (Goheen and Cook, 1959) proved that it is a grapevine leafroll associated virus (GLRaV) infection which causes their symptoms as similar to the symptoms of potassium deficiency. GLRaV 1, 2, 3, 4, 5, 6, 7, and 9 viruses have been reported in the category of grapevine leafroll viruses (Bosica et al., 1995). There are about 10 grapevine leafroll associated viruses (Martison, 2008) and grouped according to their identity. Most of the GLRaV viruses are classified under the family closteroviridae and belongs to the genera of *Closterovirus* and *Ampelovirus*. They are rod shaped, electron microscopic ranges from 1, 250 to 2, 200 nm in length (Golino et al., 2008), 10-12 nm in diameter and highly flexous (Rayapati et al., 2008). These infections are caused not only by single virus but mixture of viruses (Martelli, 1993). GLRaV-3 with genome size of 17,919 nucleotides (nt) is claimed as the second largest genome in the RNA virus category. GLRaV-1 and GLRaV-2 has the genome size of 17, 647 and 16, 494 nucleotides, respectively (Rayapati et al., 2008). Among these category of viruses, the GLRaV-1 and 3 were found to cause the maximum damage to the crops and had an economic impact (Cabaleiro, 1999). GLRaV viruses from 1-8 are considered to be non-sap transmissible phloem limited viruses. Leaf roll disease is capable of causing 40-60% decrease in grapevine yield (Peake et al., 2004). These viruses colonize and reproduce in phloem tissues of grapevines. Infection in the vascular tissues caused decrease in flow of nutrients and supplements to all parts of the grapevines which in turn resulted into lower vigour, low accumulation of sugars and problems in fruit maturity (Fuchs, 2007). GVA (A, B, D) virus classified under the genera *vitivirus.* Infection of grapevine leaf roll virus decreased the cane growth, root growth and sugar content in grapes (Over de Linden, 1970). Whereas, proper sanitation to the viruses infected grapevines were reported to increase the grapevine vigour; accumulation of anthocyanins and SSC in the grape berries; and had no effect on yield and acidity (Guidoni et al., 1997) .

2.3.1. Transmission

GLRaV and GVA are mostly spread through cultural practices such as infected rootstock and scion, vectors like mealy bugs and other soft scale insects (Sforza et al., 2003). Mealy bug species that act as causative agents for the grape leafroll diseases are found to be *Planococcus citri*, *Pseudococcus longispinus*, *P. affinis, P.*

calceolaria, P. comstocki, P. viburni, Heliococcus bohemicus (Rayapati et al., 2008). Mealy bugs are hemi metabolous and phloem feeding insects. After acquiring the virus, mealy bugs have the capability to retain it for 12 hours to 5 days (Charles et al., 2006). They usually feed on leaves, shoots, and fruit and sometimes on rootstocks. They can spread virus as airborne (Charles et al., 2006) and First instars mealy bugs are more prominent in spreading GLRaV-3 than other types when compared with adults (Golino et al., 2002).

2.3.2. Symptoms and impact

Grapevine virus A (GVA) mostly follows the symptom of Kober stem grooving syndrome (Credi and Giunchedi, 1996). The infection leads to swelling at grafted union and mortality of the vines. Grapevine virus B (GVB) shows the symptoms of corky bark disease which leads to further growth failure in the grafted part. The other symptoms are same as that of leafroll symptoms. Generally the viruses belong to closteroviridae family seem to show similar symptoms in the virus infected grapevines. Infected vines show less vigour in growth and mature leaves seems to be cupped. Whereas, the red fruit variety shows reddening of leaves while the main veins of the leaves remains green. White cultivars exhibit downward rolling of leaves and yellowing of the rolled leaves (Fuchs, 2007). The leaves of the infected vines turn brittle and thicken (Goheen et al., 1959). Phloem infection delayed sugar accumulation in berries, reduced accumulation of anthocyanins which leads to poor berry colour and delayed fruit maturity. This also makes grapevines susceptibility to adverse climatic conditions resulting in high mortality of the infected vines (Fuchs, 2007).

2.3.4. Occurrence

Grapevine leafroll associated virus is found in all the major grapevine growing areas of the world. The countries affected by the grapevine viruses includes New Zealand, Australia, Portugal, Italy, Spain, Germany, U.S.A, and France and Portugal (Hoefert and Gifford, 1967). The most affected cultivars are ‘Thompson seedless’, ‘Muscat Alexandria’, ‘Mission’, ‘Emperor’ (Goheen et al., 1959) ‘Mission’ (Over de Linden, 1970), ‘Burger’ fruit (Kliewer, 1976), ‘Sultana’ (Hale and Woodham, 1979), ‘Pinot noir’ (Zimmermann, 1990), ‘Riesling’ and ‘Zinfandel’ (Wolpert and Vilas, 1992), ‘Albana’, ‘Trebbiano’, ‘Romagnolo’ (Credi and Babini, 1997) and ‘Cabernet franc’

(Kovacs et al., 2001). In WA, occurrence of GLRaV-9 was first identified in 'Chardonnay' (Peake et al., 2004), followed by 'Merlot' (Charles et al., 2006) and 'Crimson Seedless' grapes (Brar et al., 2008). A new putative grapevine leafroll disease was identified and named as Carnelian virus in U.S.A by (Ghanem-Sabanadzovic, 2010).

2.3.5. Detection

Graft indexing was done with black fruited grape cultivars as indicator plants such as 'Mission', 'Cabernet Sauvignon', 'Pinot Noir', 'Cabernet Franc' and 'Barbera'. Herbaceous indexing has been practised by inoculating the most susceptible plant mechanically in to the healthy plant. Graft indexing with chip and cleft budding can detect only the severe infections of the virus rather than minor infections. Time consumption and sensitivity became the major limiting factor for this method (Martelli, 1993). Enzyme linked immunosorbent assay (ELISA) is another method followed in detection of virus which is based on their reaction of specific antibodies to the virus protein coat. Still it has got a limitation to describe the low level of infection of virus (Krake et al., 1999; Weber et al., 1993). Double stranded RNA (dsRNA) is another indexing method followed for identification of grapevine diseases (Saldarelli et al., 1994). Reverse transcriptase polymerase chain reaction (RT-PCR) technique is used to detect grapevine virus incidence from tissues of grapevine (Krake et al., 1999). RT-PCR technique is more sensitive than ELISA and is used in many cases of leafroll viruses (Peake et al., 2004). However, due to low cost and user friendly procedure, ELISA is routinely used for detection of virus (Sforza et al., 2003).

2.3.6. Influence of grapevine viruses on quality of grapes

Grapevine leafroll viruses are reported to negatively impact colour, anthocyanin content of berries, vine growth, sugar accumulation, fruit yield, TA, SSC. Additionally, grapevine leafroll viruses infected vines were found to be more susceptible to other infections and adverse climatic conditions (Alley et al., 1963; Credi and Giunchedi, 1996; Fuchs, 2007; Martelli, 1993). 'Nebbiolo' clone was claimed to increase vine vigour and SSC of juice by heat treating the vines infected with grapevine leafroll associated virus type 3 and GVA viruses (Guidoni et al., 1997). GLRaV-3 infected 'Albarino' vines have been reported to reduce SSC, and

increase TA (Cabaleiro et al., 1999). Over years there was no clear study describing the influence of viral infection on grape berry firmness. ‘Emperor’ clones with the mild strains of leafroll virus showed better performance with appearance and crispness of berries, had high yield without delay in ripening. Viral sanitation in grapevine fan leaf virus (GFLV) and GLRaV-1 infected grapevine cultivars ‘Manto Negro’ and ‘Moll’ were reported to improve must quality, but decreased the yield (Cretazzo et al., 2009). Selective elimination of viruses such as GLRaV-1, GLRaV-2, GLRaV-3, along with GVA and Grapevine fleck virus (GFKV) from infected clones lead to increase in fruit yield, sugar concentration, and vigour in ‘Chardonnay’ grapes (Komar et al., 2007). Clones developed in WA with inoculation of mild isolates of GLRaV and GVA viruses have been reported to produce berries heavier than virus free clones but SSC and TA in mature berries were not influenced. However, there was reduction in berry colour development in 314, and 306 clones of ‘Crimson seedless’ grapes (Brar et al., 2008). The GLRaV-3, 9, rupestris stem pitting virus (RSPaV) and GVA infected clone 314 of ‘Crimson Seedless’ grapes have been reported to produce berries with higher crispiness and better flavour than virus free standard clone and scored higher for overall acceptability even after one month of cold storage (Jayasena and Cameron, 2008). The Influence of grapevine and leafroll viruses on the composition and quality of grape berries is summarised in Table 2.1.

Table 2.1 Effect of grapevine leafroll virus on yield and quality in different cultivars of grapes.

Cultivar	Type of virus	Place	Influence or impact on grapevines	Reference
'Pinot noir' clone 114 scion	GLRaV-1, 2, 3, RspaV	U.S.A	Reduced SSC, anthocyanins, total phenolics	Jungmin and Martin (2009)
'Crimson Seedless'-clone 314	GLRaV -9, GLRaV- 3, RspaV, GVA + Ethephon	Australia	Increased TA, sensory scores for sweetness, crispness, flavour, berry colour and overall acceptability	Jayasena and Cameron (2008)
Clone- 306	GVA, GLRaV-3, GLRaV 5, RspaV 9 + Ethephon		Reduced crispness but improved berry colour	
'Crimson seedless' Clone 314 Clone 306	GLRaV 3+GLRaV 9+GVA GLRaV-3+GLRaV5+GLRAV 9+GVA	Australia	Increases the berry weight, reduces berry skin colour, reduced anthocyanin accumulation	Brar et al. (2008)
'Cabernet franc', 'Lemberger'	GLRaV -1, GLRaV- 2, GLRaV - 3	California	Lowered SSC accumulation, TA, increasesed juice pH	Martison and Fuchs (2008)
'French American' hybrid	GLRaV -3, GFKV	U.S.A	Increasesed TA, lowered average berry weight	Kovacs et al. (2001)
'Albarino'	GLRaV -3	Spain	Lowered SSC, pH, increasesed TA	Cabaleiro et al. (1999)

Ethephon (2-chlorethylphosphonic acid)

Table 2.1 continued. Effect of grapevine leafroll virus on yield and quality in different cultivars of grapes.

Cultivar	Type of virus	Place	Influence or impact on grapevines	Reference
'Trebbiano', 'Albana', 'Romagnolo'	GFLV, GLRaV-3, RW(KSG+RSP)VM	Italy	Decreased yield, lowered SSC in infected vines.	Credi and Babini (1997)
'Grignolino' & 'Nebbiolo'	GLRaV– 1+GVA GLRaV– 3+GVA	Grisliasco, Italy	Decreasedberry weight, no effect was noted on SSC. Increased TA, lowered SSC, no effect was seen on berry weight and tartaric acid.	Guidoni et al., (1997)
'Riesling' 'Zinfandel'	LR	California	Delayed sugar accumulation, lowered SSC, TA and pH not affected.	Wolpert and Vilas (1992)
'Sultana'	Not shown	Australia	Reduced SSC, increased TA, increased pH, and levels of malate, tartarate, Potassium in berries pH, high levels of malate, tartarate	Hale and Woodham(1979)
'Burger'	Not recorded	California	TA, malate and tartarate acids were high, SSC was less.	Kliewer and Lider (1976)
'Burger'	Not defined	California	Reduced fruit yield, SSC, increased TA	Lider et al., (1975)

Table 2.1 continued. Effect of grapevine leafroll virus on yield and quality in different cultivars of grapes.

Cultivar	Type of virus	Place	Influence or impact on grapevines	Reference
'Mission'	Not known	New Zealand	Reduced cane and root growth, fruit yield, sugar, pigment concentration.	Over de Linden et al., (1970)
Baco 22A	Not known	New Zealand	Reduced yield and Sugar	
'Burger','FrenchColombard', 'Zinfandel', 'Cabernet Sauvignon', 'Pinot St. George'	No information	California U.S.A	Slowedthe development of vines, lowered sugar content, and yield was reduced to one third.	Goheen and Cook (1959a)

2.4. Post-harvest physiology

2.4.1. Harvest and maturity

Maturity refers to the stage at which the fruit or vegetable reaches the state of harvest, and ripe is ready to consume (Michael, 2002). Grape is a non-climacteric fruit with low account of physiological activity at maturity. They do not continue to ripen after harvest; hence they should be harvested at correct stage of maturity as suitable for consumption. Maturity indices for grapes include size of the berries, colour, SSC, TA, and SSC: acid ratio. Since these parameters are cultivar specific and depends on the environment (Guelfat-Reich and Safran, 1971). Harvest maturity depends on the number of berries and colour of the entire clusters in the bunch. Colour is the main criterion in the case of pigmented varieties (Nelson, 1979). Palatability of the grapes increases with SSC: acid ratio (Winkler et al., 1974).

2.4.2. Grading, packing and pre-cooling

Most of the table grapes are handpicked which allows removal of poor quality berries with insects, diseases, sunburn to improve cluster appearance (Creasy and Creasy, 2008). In some cases trimming of the clusters were done in the vineyard at the time of harvest. First quality sorting that is colour sorting, trimming for presentation and, packing of clusters is carried out at the field level. Water loss is one of the serious problems in post-harvest handling phase which leads to weight loss, stem browning, berry shatter and shrivelling of berries. Cooling delays is the main reason for these problems, hence cooling should be done within 5-6 hours after harvest (Crisosto et al., 2001). Cooling of fruit and vegetables are done by different methods such as room cooling, forced-air cooling, hydro cooling, package icing and vacuum cooling (Kader, 2002). Rapid cooling is very important in the case of table grapes since stem browning is caused due to delayed cooling were reported by Crisosto et al. (2001).

2.4.3. Post-harvest pathology

During the storage life of grapes there are incidences of infection of grapes by microbial flora. Among them grey mould is most destructive disease. This pathogen can survive at a temperature of – 0.6°C and spread from berry to berry. Any wound in the berry surface at the time of harvest paves the way for its infection (Crisosto et al., 2001). Sulphur dioxide (SO_2) is one of the fumigation method followed to control

the grey mould disease caused by *Botrytis cinerea*. The grapes that are fumigated with SO_2 (100 ppm) for one hr has been reported as optimum level to control the conidial infection (Smilanick and Henson, 1992). The SO_2 fumigation can be applied immediately after harvest (Hanke and Auger, 1988) or in the forced air cooling (Luvisi et al., 1992). There are different kinds of SO_2 pads available in a market, which include fast and slow release SO_2 (gaseous) phases. The higher concentration of SO_2 results in toxicity, bleaching and hairline cracking (Zoffoli et al., 2008).

2.4.4. Storage

Grapes deteriorate in storage by decay or natural senescence. Being highly perishable crop, it undergoes severe problems during post-harvest phase as there is a weight loss, colour deterioration, accelerated softening, berry shatter, rachis browning (Hardenburg et al., 1986; Litcher et al., 2008). These detrimental effects lead to quality losses and prone to berry decay while prolonging the storage time (Perkins-Veazie et al., 1992). Hence various methods such as modified atmosphere packaging (MAP) or controlled atmospheres (CA) are being employed to prolong storage life of grapes. These techniques are used as an alternative to chemical methods during transport and storage of horticultural produce (Sabir et al., 2008). It includes modification of composition of the gas in the storage rooms which involves reduction of O_2 and elevation of CO_2. In past 50 years, the use of these strategies have been reported to improve and extend the postharvest life of horticultural products and maintain quality (Kader, 2002). MAP has beneficial effects in retarding weight loss, colour changes, softening, SSC and TA concentration and maintaining quality of 'Flame Seedless' grapes till 53 days in cold storage (Martinez-Romero et al., 2003).

The changes in quality of grape berries during postharvest storage period are detailed in Table 2.2. The effects of infection of mild isolates of leaf roll virus on storage life and quality of 'Crimson Seedless' grapes are yet to be investigated.

Table 2.2 Changes in quality of grapes berries during postharvest storage.

Cultivar	Place	Treatments	Influence or impact on grapevines	Reference
'Crimson Seedless'	China	Rachis 1-2 mm from fruit was treated with hot water and chlorine @ 45°C, for 8 min was kept for 4 weeks at 5˚C	Maintained berry firmness, increased scores for over all acceptability, lowered decay incidence and microbial population.	Kou et al., (2009)
'Crimson Seedless'	Western Australia	Clone 314 + ethephon 300 mg L^{-1} After 1 month in cold storage	Increased sensory scores for berry crispiness, flavour, over all acceptability, no effects on SSC, increased TA	Jayasena and Cameron (2008)
'Aledo'	Spain	MAP (polypropylene) with anti-microbial compounds (eugenol, thymol, carvacrol) stored @ 1˚C for 56 days at 90% relative humidity	Significantly retarded berry decays, colour changes, weight loss, softening, increased in SSC, SSC: acid ratio, over all sensory quality was improved	Guillen et al.,(2007)
'Crimson Seedless'	California	ABA @veraison 150 or 300 mg L^{-1} after 60 days of cold storage @ 0˚C, 85% RH	Maintained berry firmness, retarded berry weight loss, decay incidence, and berry shatter, improved visual appearance, maintained rachis quality	Cantin et al.,(2007)

Table 2.2 continued. Changes in quality of grapes berries during postharvest storage.

Cultivar	Place	Treatments	Inference	Reference
'Superior Seedless'	Spain	MAP without SO_2 pads for 7 days at 0˚C, followed by 8˚C+2 days at 20˚C.	After shelf life berries were good in visual appearance, crunchiness, no remarkable changes with berry colour, berry firmness, SSC, TA were inferred.	Artes-Hernandez et al.,(2006)
'Aledo'	Spain	So_2 generators with slightly CO_2 enriched atmosphere in a cardboard box 2±1°C 80-85% RH later for a period of 4 days at 20˚C	Loss of weight, texture, colour were delayed, glucose, fructose, sucrose remains unaffected, levels of tartaric and citric acids showed a slight increase.	Pretel et al., (2006)
'Crimson Seedless'	Spain	MAP with combination of 0.5 mL Eugenol, thymol, or menthol stored for 35 days @ 1˚C	Delayed weight loss, colour changes, maintained berry firmness, retards SSC: acid ratio delayed rachis deterioration and decays.	Valverde et al.,(2005)
'Kyoho'	China	CA 80% O_2 or 40% O_2 + 30% CO2 (MAP) stored for 60 days at 0˚C in 95% relative humidity followed by 5 days in air at 20˚C	No significant changes were noted for berry firmness, springiness, chewiness, flavours, reduced fruit decay, berry drop, rachis browning, weight loss, delayed the decrease of SSC, TA, vitamin C, weight loss	Deng et al.,(2005)
'Alphonse Lavalle'	Turkey	In polyethylene bags + 3°C, for 2 months	One bunch decayed, increased in SSC and TA	Arin and Akdemir(2004)

Table 2.2 continued. Changes in quality of grapes berries during postharvest storage.

Cultivar	Place	Treatments	Inference	Reference
'Autumn Seedless'	Spain	MAP with 15 kPaO_2 + 10 kPaCO_2 at 0˚C for 60 days followed by 7 days at 15˚C.	Maintained visual quality, flavour, texture of berries, increased SSC, no significant changes were noted for organic acids, controlled weight loss and decay development.	Artés-Hernández et al., (2004)
'Flame Seedless'	Spain	MAP Non perforated polypropylene with high CO_2 and low O_2 till 53 days in cold storage	Reduced weight loss, increased berry firmness, and sensory analyses scores such as crunchiness, juiciness sourness and good appearance.	Martinez-Romero et al.,(2003)
Red Globe	California	So_2 3.6 and 5.5 mol/kg hr at 0˚C, 95-98% RH, for 6 weeks in cold storage	No effects on berry decay were noted.	Palou et al.,(2002)
'Muscadine'	USA	20% CO_2 and 3% O_2 for 3 weeks	No appreciable damages were found.	Basiouny(1998)
'Fry', 'Summit', 'Granny Val'	Florida	SO_2 generators with polyethylene bags, stored for 6 weeks	TA, SSC, pH remained constant for all cultivars	James et al., (1999)
'Reliance', 'Saturn'	Arkansas	Dual release SO_2 pads at 2˚C for (7 and 10 weeks)	No effects were inferred on SSC, pH, colour, and flavour of the berries remained acceptable, maintained stem appearance, over all acceptability was rated for grapes in SO_2 dual release than controls.	Morris et al., (1992)

Table 2.2 continued. Changes in quality of grapes berries during postharvest storage.

Cultivar	Place	Treatments	Inference	Reference
'Muscadine'	California	20˚C, 4.5˚C, 0˚C Temperature	No changes in percent SSC, TA, sugars and organic acids were inferred.	Takeda et al.,(1983)
'Thompson Seedless'	California	2% O_2+10% CO for 4 months	Retarded berry browning and softening, delayed berry decay.	Yahia et al.,(1983)

CHAPTER 3

General material methods

3.1. Plant material

'Crimson Seedless' grapevines grown in a commercial vineyard located in Swan Valley (latitude - 31˚51′S and longitude 115˚59′E) of Western Australia were used in the experiment. The vines were 6 years old and grafted on to 'Schwarzmann' rootstock. The soil type of the experimental vineyard was classified as Herne sand (brown phase). The grapevines were spaced 3.3 m between the rows and 2.4 m between the vines. The vines were cane pruned to 6-8 canes per vine and 60-80 buds were retained per vine. Canopy was trimmed to top wire at veraison for colour improvement. All vines were sprayed with application of gibberellic acid (1 mg L^{-1}) and Ethrel® (0.65 mg L^{-1}) when panicles were at 40-80% bloom stage and two weeks after veraison respectively. Virus confirmation testing was done with bunch stalks using reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) depending upon the isolate by Waite Diagnostics University of Adelaide, South Australia.

Table 3.1 Rootstock treatments in Swan Valley plot.

Vineyard name	Year	Rootstock	Treatments
Swan Valley	2009	Schwarzmann	Control (virus free)
			Clone 3236 - LRV3 (E) + LRV3 (RT-PCR)
			Clone 3236 and 3215 - LRV3 (E) + GVA + LRV9 + LRV5
			Clone 3215 - LRV3 (E) + LRV3 (RT-PCR) + GVA + LRV9 + LRV5

E = enzyme-linked immunosorbent assay, RT-PCR = reverse transcriptase polymerase chain reaction. LRV- Leafroll virus, GVA- Grapevine virus A.

3.2. Sample collection

Grape berries were collected at various stages of berry maturation and ripening commencing from 50, 58, 64, 71 days after version (DAV) and ripe berry on 78th day DAV. Four bunches were randomly selected for sampling on either side of the

grapevine. Three berries were sampled from each bunch from the top, middle and bottom part of the same bunch. All at the sampling times, the berries free from visual symptoms of disease and physical damage were harvested. The berries were kept in polyethylene bag and placed on ice during their transportation and brought in an air conditioned car to Curtin University.

3.3. Sample collection for cold storage

Bunches were harvested randomly from the grapevines at ripe stage with minimum SSC: acid ratio of 30:1 and an acceptable Crimson red colour. The bunches free from any symptoms of fungus, moulds and any other physical damage and placed in the carton box with poly liner. Grapes were transferred to the laboratory in an air conditioned car. Grapes were pre-cooled at or below 5^{o}C with poly liner bag (430 × 420 × 200 mm × 15 μm HDPE natural) in an open carton box. SO_2 pads (Grape Tek Pty Ltd., UVASYS 460 × 260 mm dual releases) were placed to reduce spoilage before closing the liner and the closed boxes were placed in cool room. Temperature was maintained as 0 ± 0.5^{o}C. Tiny tag *Plus* Gemini Data Logger (Gemini Data Loggers, UK) using GLM software Version 2.1 was used to monitor temperature and relative humidity during the experiment. The data were recorded at each 15 min interval. One carton (2Kg) per replication was removed from cold storage for analysis at regular intervals of 28, 56, 84, 112, 140 and 168 days respectively.

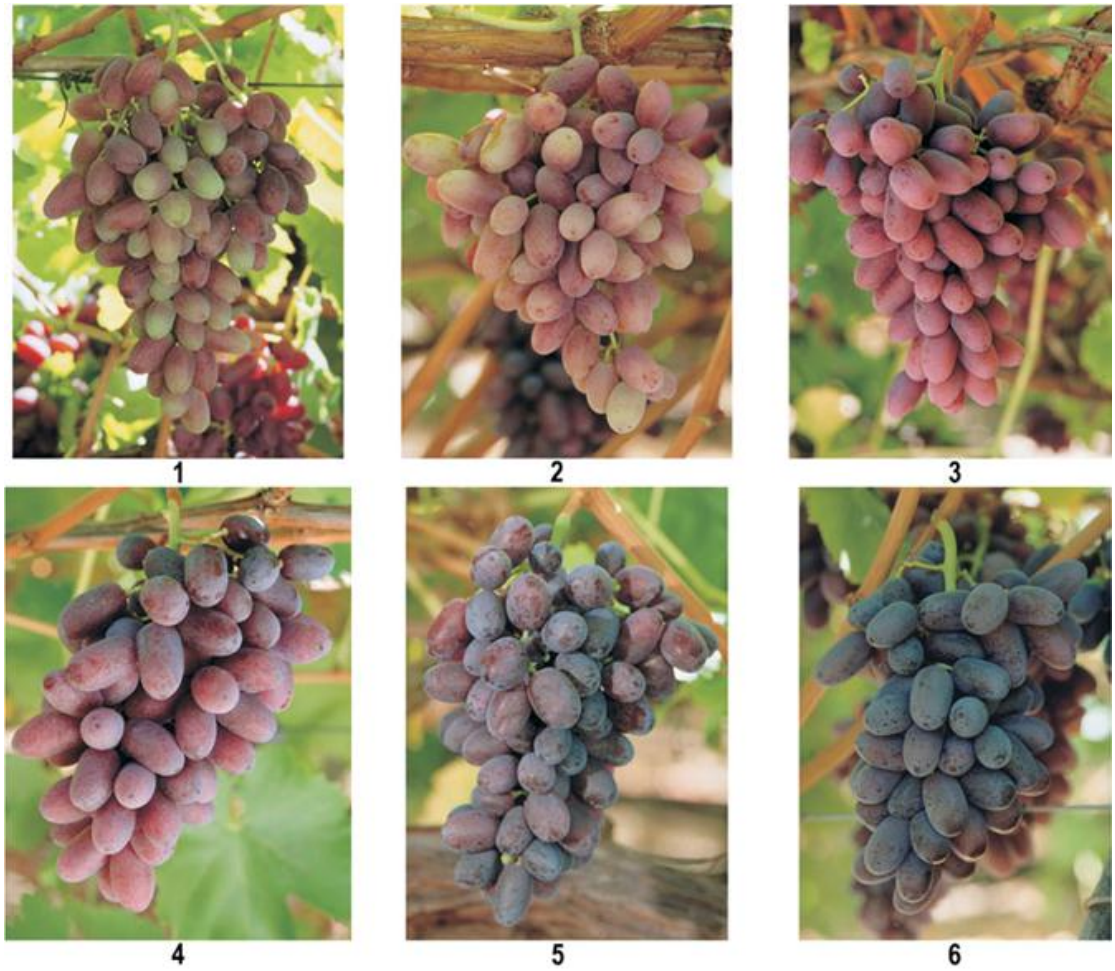

Figure 3.1 ‘Crimson Seedless’ bunch colour chart (Cameron, 2007).

3.4. Colour analysis

3.4.1. Berry colour

Berries were sampled by using bunch colour chart with rating scale from 1 to 6 (Figure 3.1). Where 1 = bunch colour with unacceptable colour (bunches with a mixture of green and poorly coloured berries), rating 2 = bunches with minimum colour of marketable acceptance, rating 3 and 4 shows the most acceptable colour (crimson red), rating 5 = light purplish black berries and 6 = Dark purplish black berries. Hence the rating score with 3 and 4 were considered for ‘Crimson Seedless’ berry sampling.

3.4.2. Commission International de L'Eclairage units (CIE) (L*, a*, b*, C* and h^o)

Twelve berries per replication were sampled for determining CIE (L*, a*, b*) values. CIE values were assessed by using a Hunter lab colour flex 45/0 spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) and expressed in the Hunter scale (L*, a*,b*, C* and h^o). The L* value represents the whiteness of the colour and it ranges from black = 0 to white = 100. Whereas a* values ranges from -60 (indicating green colour) to +60 (indicating red colour), positive b represents yellow and negative b represents blue.

3.4.2.1. Chroma

Chroma was calibrated by using the formulae $[(a^{*2}+ b^{*2})^{1/2}]$. Chroma represents colour saturation which varies from dull (low value) to vivid colour (high value).

3.4.2.2. Hue angle

Hue angle was calculated by using the formulae $H^o = \tan^{-1}$. Hue angle is used to define the changes in colour which refers to the line from the origin point to intercepting point of a^* (x- axis) and b^* (y-axis) where red purple at 0^o, yellow at 90^o, bluish green at 180^o, and blue at 270^o (McGuire, 1992). The spectrophotometer was calibrated with white and black standard tiles before recording the values for berries as shown in manufacturers manual.

3.5. Texture analysis

Textural properties of grape berry were determined during maturation, ripening and following different storage period using a texture analyser (TA Plus, AMETEK Lloyd Instruments Ltd., West Sussex, and UK) interfaced with a personal computer using Nexygen® software. The software installed in the texture machine converts the mechanical properties of the data in to a graph (Figure 3.4). A 2.0 mm Magness-Taylor probe, with a 500 N load cell on, has been used to puncture the grape berry. The berry was placed horizontal to the plane surface on the plate. This probe is suitable to puncture the berry without causing any damage to its structure (Figure 3.2 and 3.3). The berry was punctured twice pointing towards the middle of the berry with a test speed of 1.0 mm s^{-1} (Letaief et al., 2008).

Table 3.2 Measurement of berry textural characteristics.

Test	Probe	Test speed	TPA Properties
TPA	P/2 mm	1 mm s^{-1} with 50% deformation	Berry hardness (N) = maximum force required to compress the sample. Berry cohesiveness (-) = Area 2/Area 1 (extent to which the sample could be deformed prior to rupture). Berry gumminess (N) = Hardness * Cohesiveness Berry springiness (mm) = D2 (The distance and time between first bite and start of second bite).

TPA = Texture profile analyser P1 is the peak of first compression cycle. (Letaief et al., 2008; Rolle et al., 2007). Area 1 and 2 refers to the area covered under the peak.

Figure 3.2 Texture analyser linked to personal computer with Nexygen® software.

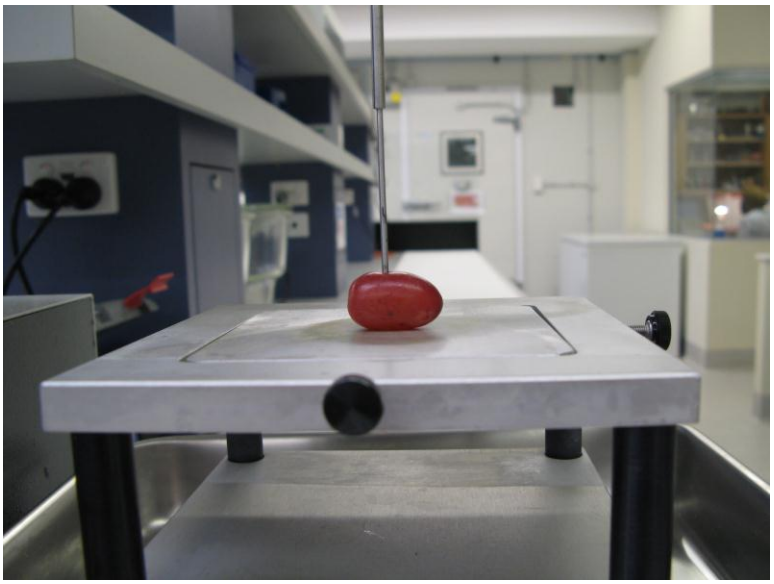

Figure 3.3 Grape puncture test using texture analyser.

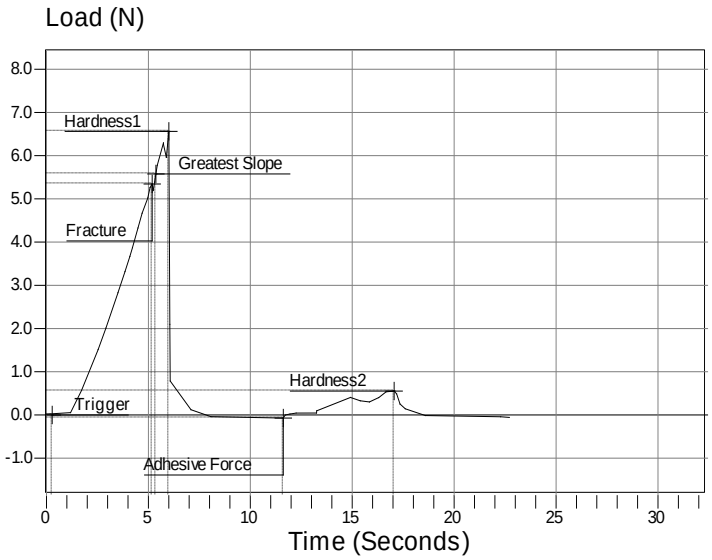

Figure 3.4 A typical texture profile analysis graph of grape berry.

3.6. Soluble solids concentration (SSC)

The juice was extracted from berries in a polyethylene bag by hand crushing. The juice was filtered through the muslin cloth into conical flask to exclude berry flesh debris. SSC was determined from the juice using a digital refractometer (Atageo PR-101, itabakshi-ku, Tokyo, Japan). SSC was expressed in per cent.

3.7. Titrable acidity (TA)

TA was determined from the juice. The juice (5ml) was titrated against 0.1N NaOH to end point pH 8.2. Phenolphthalein was used as an indicator. TA was calculated by using the formula, and expressed in per cent tartaric acid.

$$\text{TA (\%)} = \frac{0.0075 \times \text{volume of NaOH used (mL)} \times \text{Molarity of NaOH} \times 100}{\text{Volume of juice taken (mL)}}$$

3.8. SSC: acid ratio

SSC: acid ratio was calculated by dividing SSC with TA.

3.9. Sensory analysis

Sensory analysis of grape berries was carried out by untrained panel of 30 judges. The analysis was carried out in the same laboratory and the panel of judges were instructed not to discuss with each other to avoid any confusion in ratings. Rating scores were pointed on a hedonic scale with 9 points for sweetness, sourness, crispness, flavour and over all acceptability. The scale was rated according to the degree of liking of the consumers with ratings where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 =dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely **(**Jayasena and Cameron, 2008**).** The judges were advised to have crackers along with water to neutralise their tongue after tasting each sample.

3.10. Statistical analysis

The experimental data were subjected to one or two-way analysis of variance (ANOVA) using Statistical Analysis System (SAS) (SAS Institute Inc., Cary, North Carolina, United States of America (USA). Fisher's least significant differences (LSD) were calculated following a significant ($P \leq 0.05$) F-test was used to test the differences between the treatments. To ensure validity of statistical analysis all the assumptions of ANOVA were checked.

CHAPTER 4

Effects of mild isolates of grapevine viruses on the rheological properties, colour, SSC and TA during berry maturation in 'Crimson Seedless' grapes.

Summary

'Crimson Seedless' is a late maturing commercial grape cultivar. It has red and crispy berries with a sweet flavour. The effects of mild infection with Grapevine leafroll associated viruses (GLRaV-3, 5, 9) and Grapevine virus A (GVA) viruses on the textural properties of berry, colour, SCC: acid ratio during maturation in 'Crimson Seedless' grapes were investigated in 2009. Viral infected clone 3236, clone 3236 + 3215, and clone 3215 showed reduced colour development compared to the virus free control. Berries of viral infected clone 3236 + 3215 and clone 3215 showed higher berry hardness, springiness, cohesiveness and gumminess as compared to virus free control. Averaged over the ripening period, the mean SSC and SSC: acid ratio were significantly lower in the berries from the viral infected clone 3236, clone 3236 + 3215, and clone 3215 than the berries from the respective virus free controls. In conclusion, ripe berries from ~~of~~ grapevines infected with mild isolates of grapevine viruses in clone 3236 + 3215 and ~~,~~ clone 3215 showed reduced berry colour (indicated by increased a* values and decreased L*, b*, h^o angle), and SSC, increased berry springiness, gumminess, and no effect~~s~~ on TA in 'Crimson Seedless' grapes ~~than~~ compared to the virus free control.

4.1. Introduction

'Crimson Seedless' was developed by David Ramming and Ron Tarailo at the USDA Fruit Genetics and Breeding Research Unit, Fresno, CA., USA (Ramming et al., 1995). 'Crimson Seedless' is renowned for its crispy and elongated berries, excellent eating characteristics, including seedlessness and sweetness. Berry firmness, colour, sweetness, sourness and flavour are important quality parameters in selecting grape cultivars. Berry colour, sweet taste and crispiness are the overriding quality parameters in determining purchasing preference of consumers.

Grape berries typically exhibit double sigmoid growth curve commencing from berry set, growth, development, maturation and ripening stage (Mullins et al.,

1992). The berry growth in first two stages is attributed to cell division and elongation (Pratt, 1971). However, in the ripening phase, there are remarkable changes in the composition of berries such as decreased concentrations of tartaric acid, malic acid and a sudden increase in sucrose, fructose and glucose levels, as well as colour particularly in the pigmented cultivars (Coombe, 1992). Concentration of anthocyanins such as cyanidin 3-*O*-glucoside (Cn3glc), delphinidin 3-*O*-glucoside (Dp3glc), petunidin 3-*O*-glucoside (Pt3glc), peonidin 3-*O*-glucoside (Pn3glc), malvidin 3-*O*-glucoside (Mv3glc), peonidine 3-*O*-(6"-*O*-acetyl)-glucoside (Pn3Acglc), cyanidin 3-*O*-(6"-*O*-coumaryol)-glucoside (Cn3Cmglc) in the berry skin of red and black grape cultivars increases with the advancement of ripening (Mullins et al., 1992). Berry softening commences at veraison (Coombe, 1989). Berry softening enzymes such as pectinmethylesterase and polygalacturonase are responsible for reducing the pectin content in berries, which breaks the cell wall components and make the berries softer during ripening process and it varies among cultivars (Maury et al., 2009). Various factors have been reported to influence berry composition, colour, and textural properties during maturation and ripening including cultivar, environment, cultural practices, and viral infection (Smart et al., 1988; Winkler et al., 1974).

Approximately 58 viruses have been reported to cause serious damages in grapevines all over the world (Martelli, 1993). Grapevine leafroll viruses is an important graft transmissible disease of grapevines and reported to cause 66% reduction in yield (Over de Linden and Chamberlian, 1970). The infection of grapevine leafroll viruses negatively impacts growth, yield and quality characteristics of the berry (Alley et al., 1963; Goheen et al., 1959). The losses caused by grapevine leafroll associated viruses have been reported to be influenced by various factors such as cultivar, environment, and mixture of viruses (Guidoni et al., 2000; Lider et al., 1975; Wolpert and Vilas, 1992). There are about nine serologically defined grapevine leafroll viruses found in Australia (Peake et al., 2004). GLRaV-1 and 3 are considered to be most virulent among the prevailing groups. The negative effects of grapevine leafroll viruses on composition of berries have been reported in various varieties of grapes. Mild isolates of the leafroll (LR 108) in ‘Zinfandal’ and ‘White Riesling’ showed no effects on TA of berries but showed negative effects on SSC (Wolpert and Vilas, 1992). Contrarily, in ‘Nebbiolo’ clones no significant alterations

were found in SSC, the levels of TA, malic acid, tartaric acid with the infection of GLRaV-3 and GVA (Guidoni et al., 1997). GLRaV-1, GLRaV-3 and grapevine virus A (GVA) induced a reduction in vine vigour and berry skin phenolic content in 'Nebbiolo' clones (Mannini et al., 1996). Similarly in 'Nebbiolo' clones, the lower accumulation of anthocyanin in berry skin, with the infection of GLRaV-3 and GVA than in 'Grignolino' clones (Guidoni et al., 2000). Later on, the infection of mild isolates of GLRaV-3, 5, 9, GVA viruses was found to reduce the colour of 'Crimson Seedless' berries (Brar et al., 2008). 'Emperor' grapevine infected with the mild strains of leafroll virus showed better berry appearance and crispness, as well as higher yield. Clones 306 and 314 of 'Crimson Seedless' developed by Department of Agriculture and Food Western Australia (DAFWA) (Cameron, 1984) have been excelled for fruit quality particularly larger and crispier berries than the virus free standard clone (Jayasena and Cameron, 2008). No research work has been reported on the influence of the infection of mild isolates of GLRaV-3, 5, 9 and GVA viruses on the textural properties of 'Crimson Seedless' berries during maturation and ripening.

The objective of current study was to investigate the influence of mild infection of GLRaV-3, 5, 9 and GVA viruses on textural properties of berry, colour, SCC; acid ratio during maturation and ripening in 'Crimson Seedless' grapes.

4.2. Material methods

4.2.1. Plant material

'Crimson Seedless' grapevines grown in a commercial vineyard located in Swan Valley (latitude - 31˚51′S and longitude 115˚59′E) of Western Australia were used for this investigation (Table 4.1). The vines were 6 years old and grafted onto 'Schwarzmann' rootstock. The soil type of the vineyard was classified as Herne sand (brown phase). The grapevines were spaced 3.3 m between the rows and 2.4 m between the vines. The vines were cane pruned to 6-8 canes per vine and 60-80 buds were retained per vine. The canopy was trimmed to top wire at veraison for colour improvement. To all the vines, as a normal industry practice a single spray application of gibberellic acid (1 mgL^{-1}) and ethrel® (0.65 mg L^{-1}) was applied at 40-80% bloom stage and two weeks after veraison, respectively (Cameron et al., 2004).

Virus confirmation testing was done using bunch stalks tissues by employing reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) depending upon the isolate by Waite Diagnostics, University of Adelaide, South Australia.

Table 4.1 Grapevine treatments in Swan Valley plot during 2009.

Rootstock	Treatments
Schwarzmann	Control (virus free).
Schwarzmann	Clone 3236 - LRV3 (E) + LRV3 (RT-PCR).
Schwarzmann	Clone 3236 and 3215 - LRV3 (E) + GVA + LRV9 + LRV5.
Schwarzmann	Clone 3215 - LRV3 (E) + LRV3 (RT-PCR) + GVA + LRV9 + LRV5.

E = enzyme-linked immunosorbent assay, RT-PCR = reverse transcriptase polymerase chain reaction. LRV - Leafroll virus, GVA - Grapevine virus A.

4.2.2. Sample collection

Grape bunches (four) were randomly collected from either side of the grapevine at 50, 58, 64 and 71 days after version (DAV) and at ripening on (at harvest) 78th day DAV. Three berries were sampled from each bunch from the top, middle and bottom part. At all the samplings, the berries free from visual symptoms of diseases and physical damage were harvested. The berries were stored in polyethylene bags inside ice boxes during their transportation to the laboratory in an air conditioned car.

4.2.3. Observations recorded

4.2.3.1. Berry colour

Bunches were selected using bunch colour chart with the rating scale and berries were sampled as explained in Chapter 3, Section 3.4.1.

4.2.3.1.2. Berry colour Commission International de L'Eclairage units (CIE) (L*, a*, b*, C* and h^{o})

Twelve grape berries per replication were sampled for determining of CIE L*, a*, b*, C* values, h^o angle during maturation and ripening using a Hunter lab colour flex 45/0 spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) as explained in Chapter 3, Section 3.4.2.

4.2.3.1.3. Chroma (C*)

C* values of berries were calculated as explained in Chapter 3, Section 3.4.2.1.

4.2.3.1.4. Hue angle (*h°*)

Hue angle of berries were calculated as explained in Chapter 3, Section 3.4.2.2.

4.2.3.2. Texture analysis

Twelve grape berries were used for determination of various properties of texture during maturation and ripening using a texture analyser (TA Plus, AMETEK Lloyd Instruments Ltd., West Sussex, and UK) as detailed in Chapter 3, Section 3.5.

4.2.3.3. SSC

The juice was extracted from randomnly selected berries and SSC was determined using a digital refractometer as explained in Chapter 3, Section 3.6.

4.2.3.4. TA

The juice (5ml) was titrated against 0.1 N NaoH as explained in detail in Chapter 3, Section 3.7.

4.2.3.5. SSC: acid ratio

SSC: acid ratio of the juice was calculated as explained in Chapter 3, Section 3.8.

4.2.3.6. Statistical analysis

The experimental data were subjected to one or two-way analysis of variance (ANOVA) using Statistical Analysis System (SAS) SAS (release 9.1.3, SAS Institute Inc., Cary, North Carolina, USA) as explained in Chapter 3, Section 3.10.

4.3. Results

4.3.1. Changes in berry colour during maturation and ripening

4.3.1.1. CIE L* value

Irrespective of viral infection, berry CIE L* values decreased during maturation and ripening (50, 57, 64, 71 and 78 DAV) in all the clones (Table 4.2). ($P \leq 0.05$) Significantly lower CIE L* values were recorded in control as compared to the viral infected clones (clone 3236, clone 3236 + 3215, clone 3215) at all the sampling dates (Table 4.2). CIE L* values during maturation and ripening (50, 57, 64, 71 and 78 DAV) did not differ significantly among viral infected clones 3236, clone 3236 + 3215 and clone 3215. When averaged over berry ripening stage, the mean CIE L* values were ($P \leq 0.05$) significantly higher in viral infected clone 3236, clone 3236 + 3215 and clone 3215 than in virus free control. However, the differences in the CIE L* values among the viral infected clones did not differ significantly over ripening period. Averaged over treatments, the mean CIE L* values was ($P \leq 0.05$) significantly lower on 78 DAV as compared to the mean CIE L* values on 50, 57, 64 and 71 DAV.

Table 4.2. Changes in CIE L* values of 'Crimson Seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	26.49b	26.33b	24.24a	22.22b	18.60b	23.57b
Clone 3236	39.95a	37.82a	31.05a	29.28a	23.77a	32.38a
Clone 3236 + 3215	38.37a	35.89a	32.46a	28.47a	23.31a	31.58a
Clone 3215	38.49a	36.77a	31.98b	28.43a	24.58a	32.08a
Mean (DAV)	35.82A	34.20A	29.93B	26.96C	22.57D	
LSD ($P \leq 0.05$)	T = 1.98, DAV = 2.2, T × DAV = ns					

Decrease in L* values indicates that colour is becoming darker.

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5)**.**

4.3.1.2. CIE a* value

In all the clones, CIE a* values increased with the advancement in berry maturation and ripening, irrespective of the viral infection (Table 4.3). At ripe stage, CIE a* values of berry from the viral infected clone 3215, clone 3236 + 3215, clone 3215 were ($P \leq 0.05$) significantly lower than the virus free control values. When averaged over treatments, the mean CIE a* values of berry were ($P \leq 0.05$) significantly higher at ripe stage (78DAV) compared to 50, 57, 64 and 71 DAV. When averaged over berry ripening time, the means of berry CIE a* values were significantly higher in control when compared with the viral infected clone 3236, clone 3215 and clone 3236 + 3215 (Table 4.3).

Table 4.3. Changes in CIE a* values for 'Crimson Seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	2.77a	6.52a	6.78a	7.47a	7.56a	6.22a
Clone 3236	0.66b	2.56b	3.03b	4.73b	5.56b	3.19b
Clone3236 + 3215	-1.45c	2.43b	3.58b	5.08b	4.95b	3.05b
Clone 3215	0.07bc	2.31b	3.73b	5.12b	5.32b	3.30b
Mean (DAV)	0.52A	3.43B	4.29B	5.60B	5.84C	
LSD ($P \leq 0.05$)	T =0.87, DAV =0.98, T × DAV = ns					

Increase in a* values indicates the redness.

ns = not significant, n = 48 (4 replicates, 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq$

0.05) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.1.3. CIE b* value

CIE b* values of berry were found to decrease during berry maturation and ripening (50, 57, 64, 71 and 78 DAV) in all the clones irrespective of the viral infection (Table 4.4). Berry CIE b* values were ($P \leq 0.05$) significantly higher in the viral infected clone 3236, clone 3236 + 3215, clone 3215 during maturation and ripening (50, 57, 64, 71 and 78 DAV) compared to the virus free control values. When averaged over berry ripening time, the mean CIE b* values of berry were significantly lower in the virus free control as compared to the viral infected clone 3236, clone 3236 + 3215 and clone 3215. When averaged over treatments, the mean CIE b* values of berry were ($P \leq 0.05$) significantly lower at the ripe stage (78 DAV) compared to 50, 57, 64 and 71 DAV (Table 4.4).

Table 4.4. Changes in CIE b* values for 'Crimson Seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments(T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	8.30b	5.15b	3.08c	2.32b	1.17b	4.00b
Clone 3236	11.98a	8.70a	8.64a	7.37a	4.92a	8.32a
Clone 3236 + 3215	11.68a	8.71a	7.92ab	7.03a	4.51a	7.97a
Clone 3215	11.41a	9.16a	7.50b	5.86a	4.41a	7.67a
Mean (DAV)	10.84A	7.93B	6.79bC	5.65C	3.76D	
LSD ($P \leq 0.05$)	T =1.06, DAV =1.1, T × DAV = ns					

Decrease in -b* values indicates blueness.

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236

(LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.1.4. Chroma value (C*)

The C* values of berry decreased during maturation and ripening (50, 57, 64, 71, 78 DAV) in all the clones (Table 4.5). At initial stage of ripening 50 DAV, the berry C* values were ($P \leq 0.05$) significantly lower in the viral infected clone 3236 + 3215, clone 3215 and clone 3236 than virus free control. At ripe stage 78 DAV, berry C* values fails to show significant difference among the virus free control and the viral infected clone 3236, clone 3215 + 3236, clone 3215. When averaged over berry ripening period, the mean berry C* values were significantly lower in control than in the viral infected clone 3236, and clone 3236 + 3215. However, means of berry C* values does not differ significantly among the viral infected clone 3236, clone 3215 + 3236, clone 3215. When averaged over treatments, the mean C* values of berry were ($P \leq 0.05$) significantly lower at the ripe stage (78 DAV) compared to 50, 57, 64 and 71 DAV (Table 4.5).

Table 4.5. Changes in chroma values (C*) for 'Crimson Seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	58	64	71	80	(Treatments)
Control (virus free)	8.80b	8.43	7.48	7.82	7.81	8.07b
Clone 3236	12.05a	9.20	9.21	8.83	7.01	9.25a
Clone 3236 + 3215	11.77a	9.07	8.78	8.85	7.18	9.12a
Clone 3215	11.47a	9.52	8.41	7.95	7.90	8.91ab
Mean (DAV)	11.02A	9.05B	8.47B	8.36B	7.48C	
LSD ($P \leq 0.05$)	T = 0.86, DAV = 0.96, T × DAV = ns					

Decrease in C* values indicates darker colour (vividness).

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison. Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD),

Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.1.5. Hue angle (h^o)

Hue angle of berry were found to decrease with the advancement of maturation and ripening (50, 57, 64, 71 and 78 DAV) in all the clones, irrespective of the viral infections and the virus free control (Table 4.6). The berry hue angle was ($P \leq 0.05$) significantly lower in the virus free control when compared with the viral infected clone 3236, and clone 3236 + 3215 during maturation and ripening 50, 57, 64, 71 and 78 DAV. When averaged over berry ripening time, the mean berry hue angle were significantly lower in the virus free control than in the viral infected clone 3236, 3236 + 3215, and clone 3215. When averaged over treatments, the mean berry hue value was found to be lower in berries at 78 DAV as compared to those at 50, 57, 64 and 71 DAV (Table 4.6).

Table 4.6. Changes in hue angle (h^o) of 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	70.94b	39.48b	25.53b	17.25b	8.44b	32.32b
Clone 3236	87.00a	73.83a	70.83a	56.57a	45.03a	66.65a
Clone3236 + 3215	96.81a	73.60a	66.12a	54.41a	39.17a	66.02a
Clone 3215	89.24a	75.77a	63.18a	46.02a	37.84a	62.40a
Mean (DAV)	85.99A	65.67B	56.41C	43.56D	32.62E	
LSD ($P \leq 0.05$)	T = 7.5 , DAV = 8.45, T × DAV = ns					

Decrease in hue angle indicates darker colour.

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236

(LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.2. Changes in textural properties of berry during maturation and ripening

4.3.2.1. Berry hardness

Berry hardness decreased during maturation and ripening (50, 57, 64, 71 and 78 DAV) irrespective of the viral infection (Table 4.7). All the treatments did not significantly affect the berry hardness during maturation and ripening. When averaged over treatments, mean berry hardness was ($P \leq 0.05$) significantly higher at 50 DAV compared to the ones at 57, 64, 71 and 78 DAV. When averaged over the berry ripening time, the mean berry hardness was higher in the viral infected clone 3236 + 3215, and clone 3215 than the virus free control (Table 4.7).

Table 4.7. Changes in berry hardness values (N) of 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	4.68	4.46	4.22	4.19	3.81	4.27b
Clone 3236	4.94	4.20	4.02	4.44	4.10	4.34ab
Clone 3236 + 3215	4.98	4.63	4.36	4.51	4.42	4.59a
Clone 3215	4.95	4.67	4.31	4.58	4.50	4.60a
Mean (DAV)	4.88A	4.48B	4.43B	4.23B	4.21B	
LSD ($P \leq 0.05$)	T = ns , DAV = 0.32 , T × DAV = ns					

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3+ LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.2.2. Berry cohesiveness

In general, the berry cohesiveness values increased with the advancement of maturation and ripening (50, 57, 64, 71 and 78 DAV) in the viral infected clone 3236, and the virus free control (Table 4.8). When averaged over treatments, the mean berry cohesiveness does not show significant changes during berry maturation and ripening. When averaged over berry ripening time, the mean berry cohesiveness in the virus infected clone 3215 was ($P \leq 0.05$) significantly higher compared to the berries from virus free control and the clone 3236. The interaction between treatments and berry maturation and ripening period was found to be significant ($P \leq 0.05$) for berry cohesiveness (Table 4.8).

Table 4.8. Changes in cohesiveness (-) of 'Crimson seedless' berries during maturation and ripening influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	0.06b	0.06b	0.07b	0.08	0.08	0.07c
Clone 3236	0.05b	0.08ab	0.07ab	0.08	0.07	0.07bc
Clone 3236 + 3215	0.08a	0.09a	0.06b	0.08	0.10	0.08ab
Clone 3215	0.09a	0.08ab	0.09a	0.07	0.10	0.09a
Mean (DAV)	0.07	0.07	0.07	0.08	0.09	
LSD ($P \leq 0.05$)	T = 0.009, DAV = ns, T × DAV = 1.97					

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.2.3. Berry springiness

Berry springiness decreased during maturation and ripening period (50, 57, 64, 71, and 78 DAV) in the virus infected clone 3236 + 3215 and the virus free control

(Table 4.9). At ripe stage (78 DAV), the berry springiness were ($P \leq 0.05$) significantly higher in the virus infected clone 3236, clone 3236 + 3215 and clone 3215 than those of virus free control. When averaged over berry ripening time, the mean berry springiness does not differ significantly among the virus infected clones and the virus free control. When averaged over treatments, the mean berry springiness values were significantly higher at 57 and 64 DAV as compared to those on 50 and 71 DAV. There was a significant ($P \leq 0.05$) interaction between treatments and maturation and ripening period for berry springiness (Table 4.9).

Table 4.9. Changes in berry springiness (mm) of 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	3.28	3.02	3.15	2.93	1.87b	2.85a
Clone 3236	3.41	3.07	3.02	3.22	3.27a	3.20a
Clone 3236 + 3215	3.48	3.00	2.64	3.16	3.22a	3.05a
Clone 3215	3.42	2.54	2.52	3.30	3.37a	3.03a
Mean (DAV)	3.39A	2.90B	2.83B	3.15A	2.90AB	
LSD ($P \leq 0.05$)	T = ns , DAV = 0.9 , T × DAV = 0.80					

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.2.4. Berry gumminess

Berry gumminess decreased with the advancement of maturation and ripening (at 64 DAV until harvest) in the virus infected clone 3215 + 3236 (Table 4.10). At ripe stage, the berry gumminess values were higher in the virus infected clones 3236, clone 3236 + 3215 and clone 3215 than those of virus free control berries. When

averaged over berry ripening time, the mean berry gumminess values in the viral infected clone 3236 and clone 3215 were higher than the virus free control. When averaged over treatments, the mean berry gumminess does not differ significantly during maturation and ripening period. There was a significant ($P \leq 0.05$) interaction between treatments and berry ripening time for berry gumminess (Table 4.10).

Table 4.10. Changes in berry gumminess (N) of 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	0.31b	0.30	0.29	0.25	0.22a	0.29bc
Clone 3236	0.26b	0.29	0.26	0.33	0.26b	0.28c
Clone 3236 + 3215	0.49a	0.37	0.26	0.30	0.30b	0.34ba
Clone 3215	0.44b	0.40	0.28	0.34	0.33b	0.36a
Mean (DAV)	0.37	0.33	0.31	0.33	0.27	
LSD ($P \leq 0.05$)	T = 0.05 , DAV = ns , T × DAV = 0.1326					

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.3. Chemical quality attributes

4.3.3.1. SSC

In general, the SSC increased during berry maturation and ripening in the virus free as well as the viral infected clones. SSC in the virus infected clone 3215 was ($P \leq 0.05$) significantly higher at 57 DAV than SSC values of the virus free control. When averaged over berry ripening time, the mean SSC ($P \leq 0.05$) were significantly lower in the viral infected clone 3236, clone 3236 + 3215 and clone 3215 than the virus

free control. When averaged over treatments, the mean SSC was significantly higher at harvest ripe stage (78 DAV) as compared to those at 50, 57, 64 and 71 DAV (Table 4.11).

Table 4.11. Changes in SSC (%) in the 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	15.73	18.20a	18.90a	19.33	21.03	18.63a
Clone 3236	13.63	15.87ab	16.47b	17.80	19.57	16.67b
Clone 3236 + 3215	13.80	16.83ab	17.47ab	17.90	19.36	17.07b
Clone 3215	13.87	16.63b	17.13ab	19.30	19.97	17.39b
Mean (DAV)	14.26A	16.88B	17.49C	18.6C	20.07D	
LSD ($P \leq 0.05$)	T = 0.8 , DAV = 0.89, T × DAV = ns					

ns = not significant, n = 4 replicates.

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.3.2. TA

TA decreased with the advancement of berry maturation and ripening (50, 57, 64, 71 and 78 DAV) in the virus free control and the viral infected clones 3236, 3236 + 3215 and 3215 (Table 4.12). TA in berries of the viral infected clone 3236, clone 3236 + 3215 ($P \leq 0.05$) were significantly higher than TA in the berries of the viral infected clone 3215 and the virus free control. TA at ripe stage does not differ significantly among the viral infected clone 3236, clone 3236 + 3215, clone 3215 and the virus free control. When averaged over berry ripening period, the mean TA was ($P \leq 0.05$) significantly lower in the virus free control than in the clone 3236 but there were no significant differences in among the clone 3215, clone 3236 + 3215

and the virus free control. When averaged over treatments, the mean TA was significantly lower at ripe stage 78 DAV when compared with TA at 50, 58, 64 and 71 DAV (Table 4.12).

Table 4.12. Changes in TA (%) in the 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	0.60	0.56	0.47b	0.45	0.33	0.49b
Clone 3236	0.69	0.60	0.56a	0.48	0.40	0.55a
Clone 3236 + 3215	0.65	0.58	0.56a	0.45	0.39	0.52ab
Clone 3215	0.64	0.63	0.47b	0.46	0.39	0.51ab
Mean (DAV)	0.64A	0.59B	0.50C	0.47C	0.38D	
LSD ($P \leq 0.05$)	T = 0.03, DAV = 0.041, T × DAV = ns					

ns = not significant, n = 4 replicates.

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.3.3.3. SSC: acid ratio

SSC: acid ratio increased with the advancement of maturation and ripening (50, 57, 64, 71 and 78 DAV) in both the virus free control and the viral infected clone 3236, clone 3236 + 3215, and clone 3215. SSC: acid ratio was ($P \leq 0.05$) significantly high in the virus free control at 57 DAV compared to the viral infected clone 3236 + 3215 and clone 3215. However, SSC: acid ratio did not differ significantly among the viral infected clones. When averaged over berry ripening period, the mean SSC: acid ratio was ($P \leq 0.05$) significantly lower in the virus infected clone 3236, and clone 3236 + 3215 than in the virus free control. When averaged over treatments, the mean SSC: acid ratio was higher at ripe stage (78 DAV) compared to the SSC: acid ratios at 50,

57, 64, and 71 DAV (Table 4.13).

Table 4.13. Changes in SSC: acid ratio in the 'Crimson seedless' berries during maturation and ripening period influenced by the infection of grapevine viruses.

Treatments (T)	Days after veraison (DAV)					Mean
	50	57	64	71	78	(Treatments)
Control (virus free)	26.2	32.5a	40.5a	40.7	63.7	40.7a
Clone 3236	19.8	26.6ab	34.0ab	40.7	48.9	34.0b
Clone 3236 + 3215	21.4	29.0b	31.4b	40.1	49.6	34.3b
Clone 3215	21.7	26.7b	40.7a	41.5	51.2	36.4ab
Mean (DAV)	22.2C	28.6B	36.6B	40.7B	53.4A	
LSD ($P \leq 0.05$)	T = 5.7, DAV = 6.4 , T ×DAV = ns					

ns = not significant, n = 4 replicates.

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the Least Significant Difference (LSD), Control - virus free, Clone 3236 (LRV3 + LRV3), Clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), Clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV5).

4.4. Discussion

4.4.1. Berry colour

The decrease in CIE L*, b*, C* values, h^o angle (Tables 4.2, 4.3, 4.4, 4.5 and 4.6) during berry maturation and ripening showed that the berries had attained intense colour. This is consistent with previous report where Carreno et al., (1995) also reported that in pigmented grape the CIE L*, b*, C* values, h^o angle decreased and a* value increased with the colour development in 'Don Mariano'. In the current study, the a* values increased with colour development in both the viral infected as well as the virus free 'Crimson Seedless' berries (Table 4.3). The improvement in the colour during maturation and ripening in all the clones confirmed previous report that the stage III of grape berry growth is marked with the accumulation of

anthocyanins which lead to development of the characteristic berry colour in pigmented grapes (Coombe, 1992).

The reduction in colour development in the clones infected with grapevine leafroll virus (clone 3236, 3236 + 3215 and 3215) over the virus free control is in agreement with the earlier report (Brar et al., 2008), who also reported that grapevine leafroll infection reduced colour as a result of lower levels of Cn3glc, Dp3glc, Pt3glc, Pn3glc and Mv3glc anthocyanins. Possibly, the reduction in accumulation of anthocyanins in the berries from viral infected clones may be attributed to the regulation of activities of enzymes involved in the anthocyanins biosynthesis pathway as has previously been reported in 'Nebbiolo' grapes (Guidoni et al., 1997). GLRaV-2 and 3 infection have also been reported to reduce anthocyanin accumulation in 'Pinot Noir' grape (Lee and Martin, 2009). It is also possible that the reduction in berry colour development in the berries from virus infected clones might be due to reduced supply of photosynathates to the grape berries (Gholami, 2004). Previously, the infection with grapevine leafroll associated viruses has been reported to reduce photosynthetic activity in leaves of grapevines (Cabaleiro et al., 1999; Guidoni et al., 1997).

4.4.2. Textural properties

A decrease in the berry hardness was observed with the advancement of maturation and ripening in the virus infected as well as the virus free clones (Table 4.7). The increase in the activity of cell wall degrading enzymes during stage III of the grape berry development may have led to the reduction in berry hardness (Coombe, 1960; Coombe and Hale, 1973). Earlier, it has also been reported that softening of grape berry is due to decrease in elastic modules of pericarp cells (Coombe and Phillips, 1980). When averaged over ripening time, the mean berry hardness was higher in the viral infected clone 3215 and 3236 + 3215 compared to the virus free control. This observation is in agreement with a previous report on 'Crimson seedless' grapes in which higher berry crispness have been reported in grapevine leafroll virus infected clone 314 than control (Jayasena and Cameron, 2008). Lowest berry hardness in the virus free control (Table 4.7) may be attributed to higher SSC values compared to SSC values of virus infected clones. Similarly, Lee and Bourne (1980) reported a negative correlation between SCC and berry firmness in 'Barbera' grapes. Higher

berry springiness, cohesiveness and gumminess in the viral infected clone 3215 and 3236 + 3215 than that of the virus free control may be a contributing factor for higher berry hardness in the viral infected clones (Table 4.8, 4.9, 4.10). The exact mechanism involved in the increased berry hardness in the viral infected clone 3215, clone 3236 + 3215 compared to the virus free control warrants further investigation. Irrespective of the viral infection, the berry cohesiveness gradually increased with the advancement of berry ripening (Table 4.8). Berry cohesiveness has been reported to increase with the advancement of ripening in grapes (Le Moigne et al., 2008)

4.4.3. SSC

The increased SSC with the advancement of ripening in all the 'Crimson Seedless' clones is in agreement with the earlier reports as the accumulation of sugars in grape berries begins at ripening phase (Coombe, 1989). Lower SSC was recorded in the viral infected clones 3236, 3215 + 3236 and 3215 (Table 4.11) when compared with the virus free control during maturation and ripening; however, the differences were not significant among the viral infected clones. This may be due to reduction in supply of photosynthates in the viral infected clones as reported earlier (Cabaleiro et al., 1999; Guidoni et al., 1997). Similarly, lower levels of SSC has been reported in some grape cultivars infected with leafroll virus such as: 'Burger', 'Albana' and 'Trebbiano' (Credi and Babini, 1997; Kliewer and Lider, 1976); 'Cabernet Franc' (Woodham et al., 1983); 'St. Vincent' and 'Vidal Blanc' (Kovacs et al., 2001); 'Nebbiolo'(Goheen and Cook, 1959); 'Sultana' (Hale and Woodham, 1979); 'Mission' and 'Baco 22A' (Over de Linden and Chamberlian, 1970); 'Albarino' (Cabaleiro et al., 1999); and 'Crimson Seedless'(Brar et al., 2008).

4.4.4. TA

The decreased in TA in the berries of the viral infected clones (3236, 3236 + 3215 and 3215) and the virus free control during maturation and ripening is in agreement with earlier findings of Kluba et al., (1978). This may be due to decrease in malic acid concentration after veraison till ripe stage (Hardy, 1968). Irrespective of the maturation stage, higher mean TA was recorded in the viral infected clones over the virus free clone but the differences were significant only with the clone 3236 (Table 4.12). The findings are in agreement with the earlier reports on 'Burger' (Kliewer and Lider, 1976), 'St Vincent and Vidal Blanc' (Kovacs et al., 2001), 'Albarino'

(Cabaleiro et al., 1999), 'Emperor' (Cameron, 1984) and 'Sultana' (Hale and Woodham, 1979). High TA values in the virus infected clones may be due to higher levels of malic acid as reported in 'Crimson Seedless'(Brar et al., 2008).

4.4.5. SSC: acid ratio

Similar to the earlier reports on grape cultivar 'Basrah' (Al-Kaisy et al., 1981) and 'Cowart Muscadine' (Flora and Lane, 1979), the mean SSC: acid ratio increased during maturation and ripening in all the virus treatments, irrespective of the viral infection. The SSC: acid ratio in berries from the viral infected clones 3236, 3215 + 3236, 3215 was slightly lower than that in berries from the virus free control during entire period of maturation and ripening. This may be due to higher TA content in all the viral infected clones than the virus free control (Table 4.12) which reduced SSC: acid ratio as reported earlier in 'Crimson Seedless' grapes (Jayasena and Cameron, 2008).

In conclusion, ripe berries of the grapevine infected with mild isolates of grapevine viruses in clone 3236 + 3215, clone 3215 showed reduced berry colour development (a* values increased, L*, b* values h^o angle decreased) SSC, and berry springiness and gumminess than virus free control.

CHAPTER 5

Influence of infection of mild isolates grapevine viruses on cold storage life and quality of 'Crimson Seedless' grapes.

Summary

The effects of infection of mild isolates of grapevine leafroll associated virus (GLRaV) 3, 5, 9, grapevine virus A (GVA) on cold storage life and quality of 'Crimson Seedless' grapes were investigated. The viral infected clone 3236 + 3215 (LRV3 + GVA + LRV9 + LRV5), and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV) do not show any significant changes in SSC, TA, until 140 days cold storage when compared to the virus free control. Berry textural properties, such as hardness, gumminess, springiness, cohesiveness, sensory scores (berry flavour, crispiness, overall acceptability) were significantly higher in the viral infected clone 3236 + 3215, clone 3215 than the virus free control. In conclusion the viral infected clone 3236 + 3215, clone 3215 can be stored for 140 days at 0 ± 0.5^{o}C with acceptable colour, textural properties, and sensory parameters.

5.1. Introduction

Grapes, non-climacteric fruit, are stored at low temperature to extend their storage life and to stagger seasonal gluts (Ramprasad et al., 2004). 'Crimson Seedless' is an important red skinned grape cultivar well known for its storage life. In Western Australia, 'Crimson Seedless' grape is grown on a large scale from Geraldton (in the north) to the Margret River (in the south). Higher demand for 'Crimson Seedless' grape in both Australian and international markets has played a key role in expanding this cultivar in all the major table grape growing regions of Queensland and Western Australia (ATGA, 2010; Cameroon, 2005).

Like other fruits, quality of grapes also deteriorate during post-harvest phase such as: weight loss; stem drying and browning; berry shatter; wilting; and shrivelling (Crisosto et al., 2001). In grapes, berry softening during storage deteriorates quality and reduce disease resistance (Nelson, 1978). Grape is highly susceptible to fungal rots caused by grey mould (*Botrytis cinerea* Pers). Post-harvest decay of grape is controlled with sulphur dioxide (SO_2) fumigation in polyethylene-

lined boxes with continuous release of SO_2 from sulphur dioxide generating pads in cold storage (Luvisi et al., 1992). 'Saturn' grapes were stored successfully till 12 weeks with SO_2 pads at 2^oC (Perkins-Veazie et al., 1992). The SO_2 fumigation has been implemented in various cultivars to prevent decay incidence (Morris et al., 1992; Nelson, 1983; Sabir et al., 2008; Smilanick et al., 1990).

In the last decade, modified atmospheric packages (MAP) and controlled atmosphere (CA) have emerged as alternative strategies for the SO_2 pads to alleviate fruit decay and to extend the shelf life of fresh table grapes (Artes-Hernandez et al., 2006; Lydakis and Aked, 2003). The use of MAP has been reported to reduce weight loss, colour changes, softening and increase SSC: acid ratio in 'Flame Seedless' stored at 1^oC for 53 days (Martinez-Romero et al., 2003).

Amongst the virus and virus like agents, the grapevine leafroll associated virus (GLRaV-1) and GLRaV-3 are considered as the most virulent viruses, which has deleterious effects on grape quality (Guidoni et al., 1997). The berries from the grapevine infected with leafroll showed reduced colour development; lower levels of sugars and TA in 'Burger' grapes (Kliewer and Lider, 1976; Lider et al., 1975). The infection of GLRaV-3, GLRaV-3 + GFkV in 'Vidal Blanc' and 'St Vincent' grapes has been reported to reduce berry weight and increase TA in the juice (Kovacs et al., 2001). Inoculation of mild isolates of grapevine leaf roll associated viruses plays a moderate role in determining the quality of 'Crimson Seedless grapes' in Western Australia (ATGA, 2007). The infection of certain mild strains of grapevine leafroll viruses and grapevine viruses in 'Crimson Seedless' clone 314, clone 306 did not significantly influence the SSC, glucose, fructose, organic acids such as tartaric and malic acids (Brar, 2008). 'Sultana' clones infected with strains of leafroll virus produced bunches with larger berries but fewer berries per bunch. Whilst, 'Emperor' clones infected with mild strains of leafroll virus produced crispier, heavier and longer berries than the virus free control. The clone 306 and 314 in 'Crimson Seedless' grapes developed with infection of GLRaV 3, 5, 9 and Rupestris Stem Pitting virus (RSPaV) stored at 1^oC under cold storage for one month showed increased crispness and flavour as compared to the virus free clone (Jayasena and Cameron, 2008). The reports on the effects of the infection of these mild isolates of grapevine leafroll viruses on cold storage life and quality of 'Crimson Seedless'

grapes particularly berry texture are scant. The objective of the study was to uncover the influence of GLRaV 3, 5 and 9; and GVA viruses on cold storage life and quality of 'Crimson Seedless' grapes.

5.2. Materials and methods

5.2.1. Plant material

'Crimson Seedless' grapevines grown in a commercial vineyard located in Swan Valley (latitude 31˚51′S and longitude 115˚59′E) Western Australia. The grapevines were 6 years old and grafted on to 'Schwarzmann' rootstock. The soil type of the vineyard was classified as Herne sand (brown phase). The grapevines were spaced 3.3 m between the rows and 2.4 m between the vines. As a common industry practice all the vines were cane pruned to 6-8 canes per vine and 60-80 buds were retained per vine (Cameron et al., 2004). Canopy was trimmed to top wire at veraison for colour improvement. A single spray application of gibberellic acid (1 mgL^{-1}) was applied when panicles were at 40-80% bloom stage. An aqueous solution containing Ethrel® (0.65 mg L^{-1}) was sprayed two weeks after veraison. The confirmation of the virus in from the bunch stalk was done using reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) depending upon the isolate.

Table 5.1.

Different treatments in Swan Valley vineyard during 2009.

Rootstock	Treatments
Schwarzmann	Control (virus free).
Schwarzmann	Clone 3236 - LRV3 (E) + LRV3 (RT-PCR).
Schwarzmann	Clone 3236 and 3215 - LRV3 (E) + GVA + LRV9 + LRV5.
Schwarzmann	Clone 3215 - LRV3 (E) + LRV3 (RT-PCR) + GVA + LRV9 + LRV5.

E = enzyme-linked immunosorbent assay, RT-PCR = reverse transcriptase polymerase chain reaction.

5.2.2. Sample collection

Bunches were harvested randomly from the grapevines at ripe stage with minimum SSC: acid ratio of 30:1 and bunches had attained an acceptable crimson red colour. Bunches free from any visual symptoms of fungus, moulds and any other physical damage were selected and placed in the carton box (card board material) with polyethylene liner bag (430 × 420 × 200 mm ×15 um HDPE). They were transferred to the laboratory in an air conditioned car. Grapes were pre-cooled in the laboratory below 5^{o}C with the poly liner bag open in a carton box. SO_2 pads (Grape Tek Pty Ltd., UVASYS 460 × 260 mm dual releases) were placed in the bags to reduce spoilage before closing the liner and the boxes closed with carton lid were placed in cool room. Temperature in the cool room was maintained at 0 ± 0.5^{o}C and 95% relative humidity during storage period. Tiny tag *Plus* Gemini Data Logger (Gemini Data Loggers, UK) using GLM software Version 2.1 was used to monitor temperature and relative humidity in the cool room at 15 min interval during the entire storage period. One carton per replication was removed from cold storage for analysis at regular intervals of 0, 28, 56, 84, 112, 140 and 168 days respectively.

5.2.3. Observations recorded

5.2.3.1. Berry colour Commission International de L'Eclairage units (CIE) (L*, a*, b*, C* and h^{o})

Twelve berries per replication were randomly sampled following 0, 28, 56, 84, 112, 140 and 168 days cold storage for determining CIE L*, a*, b*, C*, h^{o} values using a Hunter lab colour flex 45/0 spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) as explained in Chapter 3, Section 3.4.2.

5.2.3.1.2. Chroma (C*)

C* values of berries were calculated as explained in Chapter 3, Section 3.4.2.1.

5.2.3.1.3. Hue angle (h^{o})

Hue angle of berries were calibrated as explained in Chapter 3, Section 3.4.2.2.

5.2.3.2. Texture analysis

Twelve berries per replication were sampled randomly following 0, 28, 56, 84, 112, 140 and 168 days cold storage to determine various textural properties of grape berries. The textural properties of grape berry were determined using a texture analyser (TA Plus, AMETEK Lloyd Instruments Ltd., West Sussex, and UK) interfaced with the personal computer using Nexygen® software as detailed in Chapter 3, Section 3.5.

5.2.3.3. SSC

The juice was extracted from randomly selected berries and SSC was determined using digital refractometer as explained in Chapter 3, Section 3.6. SSC was expressed as per cent.

5.2.3.4. TA

The juice (5ml) was titrated against 0.1N sodium hydroxide (NaOH) using phenolphthalein as an indicator of end point pH 8.2 as explained in detail in Chapter 3, Section 3.7. TA was expressed as per cent.

5.2.3.5. SSC: acid ratio

SSC: acid ratio of the berries was calculated as explained in Chapter 3, Section 3.8.

5.2.3.6. Sensory analysis

Sensory analysis of grape berries was carried out by an untrained panel comprising of 30 judges. The panel of judges were instructed not to discuss with each other to avoid confusion in ratings. Rating scores were pointed on a hedonic scale with 9 points for sweetness, sourness, crispness, flavour and over all acceptability. The scale was rated according to the degree of liking of the consumers with ratings where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 =dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely (Jayasena and Cameron, 2008). The judges were advised to have crackers along with water to neutralise their tongue after tasting each sample (Jayasena and Cameron, 2008).

5.2.3.7. Statistical analysis

The experimental data were subjected to one or two-way analysis of variance (ANOVA) using Statistical Analysis System (SAS) (SAS Institute Inc., Cary, North Carolina, USA). Fisher's least significant difference (LSD) was calculated following a significant ($P \leq 0.05$) *F*-test. All assumptions of ANOVA were checked to ensure the validity of statistical analysis.

5.3. Results

5.3.1. Changes in CIE L*, a*, b*, chroma (C*) values, hue (h^o) angle of berry during cold storage period.

5.3.1.1. CIE L* value

Berry CIE L* value declined during the cold storage period (0-168 days) in all the clones irrespective of the viral infection (Table 5.2). Berry CIE L* values were significantly lower in the virus free control than those in the viral infected clone 3215, clone 3215 + 3236 and clone 3236 following 0, 28, 56, 84, 112 days of cold storage. When averaged over cold storage period, the mean berry CIE L* values were significantly lower in the viral infected clones 3236, clone 3215 + 3236 and clone 3215 when compared with the virus free control. Averaged over treatments, the mean berry CIE L* values were significantly lower following 168 days of cold storage when compared with mean berry CIE L* values on 0, 28, 56, 84, 112, 140 days of cold storage.

5.3.1.2. CIE a* value

Berries from all the clones irrespective of the viral infection exhibited increase in CIE a* values from 0-112 days of cold storage period (Table.5.3). At 0 day (at harvest) the berry CIE a* values were significantly higher ($P \leq 0.05$) in the virus free control compared to the viral infected clone 3236, clone 3215 + 3236, and clone 3215. When averaged over cold storage period, the mean CIE a* values of berries were significantly lower ($P \leq 0.05$) in the virus free control when compared with the viral infected clone 3236, clone 3215 + 3236 and clone 3215. The mean berry CIE a* values, averaged over treatments, were significantly lower ($P \leq 0.05$) following 0, 28

and 56 days of cold storage period when compared with those stored for 84, 112, 140, and 168 days.

5.3.1.3. CIE b* value

Berry CIE b* values showed a decline during the storage period (0, 28, 56, 84, 112, 140 and 168 days) in all the clones irrespective of viral infection (Table.5.4). The berry CIE b* values were significantly lower ($P \leq 0.05$) in the viral infected clone 3236, clone 3215 + 3236 and clone 3215 than those in the virus free control. When averaged over storage period, the mean berry CIE b* values were significantly lower in the virus free control when compared with the viral infected clone 3236, clone 3215 + 3236 and clone 3215. When averaged over treatments, the mean berry CIE b* values were significantly higher ($P \leq 0.05$) following 0 and 28 days of cold storage period than the values after 168 days of storage.

Table 5.1. Changes in CIE 'L*' values of 'Crimson seedless" berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean (T)
	0	28	56	84	112	140	168	
Control (virus free)	18.6a	18.3b	17.2a	15.4b	15.4b	15.2	15.1	16.5b
Clone 3236	23.8b	22.9ab	22.5a	21.0a	21.0a	19.9	18.9	21.4a
Clone 3236 + 3215	23.3b	22.4ab	22.3a	21.0a	21.0a	19.4	17.9	21.1a
Clone 3215	24.6b	24.4a	21.5b	21.04a	21.0a	19.3	17.6	21.3a
Mean (D)	22.6A	22.0A	20.9AB	19.6BC	19.6BC	18.4CD	17.4D	
LSD ($P \leq 0.05$)	T = 1.5, Days = 2.0, T × Days = ns							

Decrease in L^* values indicates that colour is becoming darker

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$). Least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.2. Changes in CIE a* values of "Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean (T)
	0	28	56	84	112	140	168	
Control (virus free)	7.6a	7.6a	7.5	8.2	9.4	8.1	7.4	8.0a
Clone 3236	4.9b	5.5b	6.2	7.1	7.4	7.5	7.4	6.6b
Clone 3236 + 3215	5.6b	4.9b	5.0	6.3	6.8	7.6	7.7	6.3b
Clone 3215	5.3b	5.8ab	6.1	6.9	7.0	7.6	7.8	6.7b
Mean (D)	5.9C	5.9C	6.2BC	7.1AB	7.6A	7.6A	7.7A	
LSD ($P \leq 0.05$)	T = 0.9, Days = 1.2, T × Days = ns							

Increase in a* valuesindicates the redness

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.3. Changes in CIE b* values of 'Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	1.2b	1.1b	1.1b	0.9b	0.9b	0.9	0.8b	1.0b
Clone 3236	4.9a	3.8a	3.8a	3.2a	2.9a	2.8	2.8a	3.5a
Clone 3236 + 3215	4.5ab	4.0a	4.0a	3.9a	3.0a	2.9	2.0ab	3.5a
Clone 3215	4.4ab	4.2a	4.1a	3.6a	3.6a	2.9	2.4ab	3.6a
Mean (D)	3.8A	3.3AB	3.3AB	2.9ABC	2.6BCD	2.4BC	2.0C	
LSD ($P \leq 0.05$)	T = 0.72, Days = 0.9, T × Days = ns							

Decrease in -b*valuesindicates blueness

ns = not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

5.3.1.4. Hue angle (h^o)

Hue angle of berry was found to decrease in the viral infected clone 3236, clone 3215 + 3236 and clone 3215 during cold storage period (0, 28, 56, 84, 112, 140 and 168 days) (Table.5.5). The berry h^o angle was significantly lower ($P \leq 0.05$) in the virus free control when compared with the viral infected clone 3236, clone 3215 + 3236 and clone 3215 during cold storage period (0, 28, 56, 84 and 112 days). Averaged over storage period, the mean h^o angle of berries was significantly lower ($P \leq 0.05$) in virus free control when compared with the virus infected clone 3236, clone 3215 + 3236, and clone 3215. When averaged over treatments, the mean h^o angle of berries was significantly lower ($P \leq 0.05$) after 168 days of cold storage when compared with values after 0, 28, 56 and 84 days of cold storage.

5.3.1.5. C* value

The C* value of berries fluctuated during cold storage without any specific trend in all the viral infected clones and the virus free control (Table.5.6). The berry C* value did not differ significantly between the virus free control and the viral infected clone 3236, clone 3215 + 3236 and clone 3215 during cold storage period. Averaged over cold storage period, the mean C* value of berries did not differ significantly in the viral infected clone 3236, clone 3215 + 3236 and clone 3215 and the virus free control. Averaged over treatments, the mean C* value of the berries also did not differ significantly during cold storage period (0, 28, 56, 84, 112, 140 and 168 days).

Table 5.4. Changes in hue values of 'Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	8.4b	7.7b	7.9b	6.5b	5.7b	7.0	6.0b	7.0b
Clone 3236	45.0a	34.8a	31.5a	24.3ab	22.0a	19.9	20.1a	28.3a
Clone 3236 + 3215	39.2a	39.3a	38.9a	36.0a	24.6a	21.4	14.6ab	30.6a
Clone 3215	37.8a	36.4a	35.9a	29.3a	27.8a	21.1	16.9a	29.3a
Mean (D)	32.6A	29.6AB	28.5AB	24.0BC	20.1CD	17.34CD	14.4D	
LSD ($P \leq 0.05$)	T = 6.3, Days = 8.2, T × Days = ns							

Decrease in hueindicates that colour is becoming darker

ns = not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.5. Changes in CIE C* values of 'Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	7.9	7.7	7.6	8.3	9.5	8.1	7.4	8.1
Clone 3236	7.0	6.8	7.4	7.8	8.0	8.1	7.9	7.6
Clone3236 + 3215	7.2	6.4	6.4	7.7	7.5	8.2	8.0	7.3
Clone 3215	7.2	7.3	7.5	7.8	7.9	8.3	8.1	7.8
Mean (D)	7.3AB	7.03B	7.2AB	7.9AB	8.2A	8.2A	7.9AB	
LSD ($P \leq 0.05$)	T = 0.8, Days = 1.3, T × Days = ns							

Decrease in C* values indicates that colour is becoming darker (vividness)

ns = not significant, n = 40 (4 replicates and 10 berries per replication). ns= not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

5.3.2. Changes in textural properties of berry during cold storage.

5.3.2.1. Berry hardness

The berry hardness decreased with the extension of cold storage period in the virus free control and the viral infected clone 3236 (Table.5.7). The berry hardness was significantly higher ($P \leq 0.05$) during cold storage period in the viral infected clone 3236 + 3215 and clone 3215 as compared to the viral infected clone 3236 and virus free control. Averaged over storage period, the mean berry hardness was significantly higher ($P \leq 0.05$) in the viral infected clone 3215 + 3236 and clone 3215 as compared to the mean berry hardness in the virus infected clone 3236 and the virus free control Averaged over treatments, the mean berry hardness significantly reduced ($P \leq 0.05$) following 140 and 168 days of cold storage compared to 0 to 112 days of cold storage.

5.3.2.2. Berry cohesiveness

In general, berry cohesiveness decreased with extended cold storage period up to 168 days irrespective of the viral infection (Table.5.8). Berry cohesiveness were significantly higher ($P \leq 0.05$) in the viral infected clone 3236 + 3215 clone 3215 as compared with the virus free control and the virus infected clone during cold storage period 56, 112 and 168 days. When averaged over cold storage period, the mean berry cohesiveness was significantly higher ($P \leq 0.05$) in the viral infected clone 3236 + 3215 and clone 3215 when compared with mean berry cohesiveness in the virus free control and viral infected clone 3236. Averaged over treatments, the mean berry cohesiveness values reduced significantly ($P \leq 0.05$) after 112, 140 and 168 days compared to 0, 28 and 56 days of cold storage.

5.3.2.3. Berry springiness

Berry springiness decreased with the extended cold storage period 0, 28, 56, 84, 112, 140 and 168 days in the viral infected clone 3236, clone 3215 + 3236, clone 3215 and the virus free control (Table.5.9). Berry springiness values were significantly higher ($P \leq 0.05$) in the virus infected clone 3236, clone 3215 + 3236, and clone 3215 as compared to the virus free control up to 140 days of cold storage. When averaged over storage period, the mean berry springiness values were higher in the

viral infected clone 3236, clone 3215 + 3236 and clone 3215 when compared with mean berry springiness in the virus free control.

5.3.2.4. Berry gumminess

Berry gumminess values declined with extended cold storage period in all the clones including virus free control and the viral infected clone 3236, clone 3215 + 3236 and clone 3215 (Table.5.10). Berry gumminess values were significantly higher ($P \leq 0.05$) in the viral infected clone 3236 + 3215 and clone 3215 when compared with the values obtained from virus free control after 56, 140 and 168 days of cold storage. Averaged over cold storage period, the mean berry gumminess values were significantly higher ($P \leq 0.05$) in the viral infected clone 3215 and clone 3236 + 3215 compared with mean berry gumminess in the virus free control and viral infected clone 3236.

Table 5.6. Changes in 'Crimson seedless' berry hardness (N) during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	3.8	3.5b	3.0b	2.9	2.3b	2.0b	2.0b	2.8c
Clone 3236	4.0	3.6b	3.2b	2.7	2.7b	2.4b	2.1b	3.0b
Clone 3236 + 3215	4.4	4.4a	4.4a	4.3	4.1a	3.1a	3.0a	4.0a
Clone 3215	4.5	4.4a	4.4a	4.3	4.2a	3.2a	3.1a	4.0a
Mean (D)	4.2A	4.0AB	3.7BC	3.5CD	3.3D	2.7E	2.6E	
LSD ($P \leq 0.05$)	T = 0.2 , Days = 0.3 , T × Days = ns							

ns = not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison. Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.7. Changes in 'Crimson seedless' berry cohesiveness during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	0.08	0.07bc	0.06b	0.05	0.05b	0.03	0.03b	0.05b
Clone 3236	0.08	0.06c	0.06b	0.06	0.05b	0.04	0.03b	0.05b
Clone 3236 + 3215	0.08	0.08ab	0.08a	0.08	0.07a	0.07	0.07a	0.08a
Clone 3215	0.10	0.09a	0.08a	0.08	0.08a	0.07	0.07a	0.08a
Mean (D)	0.08A	0.08AB	0.07AB	0.07BC	0.06CD	0.05DE	0.05E	
LSD ($P \leq 0.05$)	T = 0.06, Days = 0.009 , T × Days = ns							

ns = not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.8. Changes in 'Crimson seedless' berry springiness (mm) during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	1.8b	1.8b	1.8b	1.4b	1.4b	1.4b	1.4	1.6b
Clone 3236	3.4a	3.1a	3.1a	3.1a	3.0ab	3.0a	2.9	3.1a
Clone 3236 + 3215	3.2a	3.2a	3.1a	3.1a	3.0a	2.9a	2.7	3.0a
Clone 3215	3.4a	3.2a	3.2a	3.1a	3.0a	3.0a	2.9	3.1a
Mean (D)	2.9	2.8	2.8	2.6	2.6	2.6	2.5	
LSD ($P \leq 0.05$)	T = 0.5, Days = ns, T × Days = 0.8							

ns = not significant, n = 48 (4 replicates , 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.9. Changes in 'Crimson seedless' berry gumminess (N) during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	0.30	0.20	0.17b	0.15	0.15	0.16b	0.14c	0.18c
Clone 3236	0.25	0.25	0.25ab	0.20	0.20	0.18ab	0.15bc	0.21b
Clone 3236 + 3215	0.30	0.29	0.28a	0.25	0.25	0.25a	0.26a	0.27a
Clone 3215	0.33	0.30	0.29a	0.26	0.26	0.25a	0.25ab	0.28a
Mean (D)	0.29A	0.26AB	0.25ABC	0.22BC	0.21BC	0.21C	0.20C	
LSD ($P \leq 0.05$)	T = 0.04, Days = 0.05, T × Days = ns.							

ns = not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

5.3.3. Changes in SSC, TA and SSC: acid ratio during cold storage period.

5.3.3.1. SSC

SSC were found to increase in the virus free control, viral infected clone 3236 + 3215 and clone 3215 with the extension in cold storage period from 0 to 168 days (Table.5.11). In contrast the SSC in the viral infected clone 3236 decreased on 56-168 days of cold storage. The SSC were significantly higher ($P \leq 0.05$) in the virus free control when compared with the viral infected clone 3236, clone 3215 + 3236 and clone 3215 on 56, 84, 112, 140 and 168 days of cold storage. When averaged over cold storage period, the mean SSC as significantly lower ($P \leq 0.05$) in the viral infected clone 3236, clone 3215 + 3236 and clone 3215 than the virus free control. However, the mean SSC in the viral infected clone 3236 was significantly lower ($P \leq 0.05$) when compared with the mean SSC in the viral infected clone 3215 and clone 3236 + clone 3215. When averaged over treatments, the mean SSC was significantly lower ($P \leq 0.05$) at 0 day at harvest when compared with the mean SSC on 140 and 168 days cold storage. The interaction between treatments and cold storage period was found to be significant ($P \leq 0.05$) for SSC.

5.3.3.2. TA

The TA in juice differ significantly ($P \leq 0.05$) in berries after 56, 140 and 168 days of cold storage in all clones including the virus free control and the viral infected clone 3236, clone 3236 + 3215, and clone 3215 (Table.5.12). When averaged over cold storage period, the mean TA was significantly higher in the berries from viral infected clone 3236 when compared with the mean TA in the virus free control and the viral infected clone 3215 + 3236 and clone 3215.

5.3.3.3. SSC: acid ratio

The SSC: acid ratio in the viral infected clone 3236 decreased between 112 and 168 days of cold storage period (Table.5.13). There was a significant increase ($P \leq 0.05$) in SSC: acid ratio in the viral infected clone 3215 from 84 to 168 days of cold storage. The SSC: acid ratio was significantly higher ($P \leq 0.05$) in the virus free control and the viral infected clone 3215 + 3236 and clone 3215 than the viral infected clone 3236 during 0 to 168 days of cold storage. When averaged over cold storage period, the mean SSC: acid ratios were significantly higher ($P \leq 0.05$) in the

virus free control and the viral infected clone 3215 and clone 3215 + 3236 as compared to mean SSC: acid ratio in the viral infected clone 3236. When averaged over treatments, the mean SSC: acid ratio does not differ significantly during (0 to 168 days) cold storage.

Table 5.10. Changes in SSC (%) of 'Crimson seedless' berries during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	20.0a	21.9	22.5a	23.0a	26.4a	26.5a	26.7a	24.0a
Clone 3236	19.6b	20.0	17.9c	17.0b	15.7c	15.4c	14.7c	17.2c
Clone 3236 + 3215	19.4b	20.0	20.2b	20.3b	20.6b	21.4b	22.4b	20.6b
Clone 3215	20.0ab	20.0	20.1b	20.2b	21.1b	21.9b	21.9b	20.7b
Mean (D)	20.0B	20.5AB	20.7B	20.1B	21.0AB	21.3A	21.4A	
LSD ($P \leq 0.05$)	T = 0.8, Days =1.1, T × Days = 2.2.							

ns = not significant

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.11. Changes in TA (%) of 'Crimson seedless' berries during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	0.33	0.32	0.32b	0.31	0.32	0.34ab	0.34b	0.32b
Clone 3236	0.40	0.41	0.39a	0.35	0.37	0.42a	0.44a	0.40a
Clone 3236 + 3215	0.32	0.32	0.31b	0.31	0.30	0.28b	0.28bc	0.30b
Clone 3215	0.36	0.35	0.37ab	0.34	0.32	0.30ab	0.27c	0.33b
Mean (D)	0.35	0.35	0.35	0.33	0.33	0.34	0.33	
LSD ($P \leq 0.05$)	T = 0.03, Days = ns, T × Days = ns.							

ns = not significant.

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.12. Changes in SSC: acid ratio of "Crimson seedless' berries during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	70.0a	67.0b	71.2a	75.1a	84.1a	78.2a	79.3a	75.0a
Clone 3236	49.3b	51.0a	46.5b	48.8b	42.5b	37.1b	33.3b	44.1c
Clone 3236 + 3215	60.5a	62.4b	65.2a	65.5a	69.1a	85.7a	81.1a	70.0ab
Clone 3215	56.1a	56.9b	54.9b	64.2a	68.7a	73.3ab	83.4a	65.4b
Mean (D)	60.0	59.3	59.5	63.4	66.1	68.5	69.3	
LSD ($P \leq 0.05$)	T = 7.9, Days = ns, T × Days = ns.							

ns = not significant, n = 48 (4 replicates and 12 berries per replication).

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

5.3.4. Changes in sensory analysis parameters of 'Crimson seedless' berries during cold storage.

5.3.4.1. Sweetness

Sensory analysis scores for sweetness were found to decrease in the berries of the virus infected clone 3236 following 0, 28, 56, 84, 112, 140 and 168 days of cold storage (Table.5.14). Sweetness scores does not show significant change in the viral infected clone 3236 + 3215, clone 3215 and the virus free control during 0-168 days of cold storage. Sweetness scores were significantly higher ($P \leq 0.05$) in the virus free control than those of the virus infected clone 3215 on 84, 112 and 168 days in cold storage. When averaged over cold storage period, the mean sweetness scores were significantly higher ($P \leq 0.05$) in the virus free control, when compared with the mean sweetness scores in the viral infected clone 3215, clone 3236 + 3215 and clone 3236. When averaged over treatments, the mean sweetness scores were found to be lower at 168 days of cold storage than those of berries stored for 0, 28, 56, 84 and 140 days in the cold storage.

5.3.4.2. Sourness

Sensory analysis score for sourness did not show significant difference during 0 to 168 days of cold storage in all clones, irrespective of the virus infection (Table.5.15). When averaged over cold storage period, the mean sourness scores were significantly higher ($P \leq 0.05$) in the virus infected clone 3215 when compared with mean sourness scores of the viral infected clone 3236 and clone 3215 + 3236.

5.3.4.3. Berry crispiness

The berry crispiness decreased during cold storage (0- 168 days) in all the clones irrespective of the viral infection and the virus free control (Table.5.16). The decrease in the berry crispiness score was more pronounced in the virus free control when compared with the viral infected clone 3236, clone 3236 + 3215 and clone 3215. The berry crispiness score was significantly higher ($P \leq 0.05$) in the virus infected clone 3236 + 3215 and clone 3215 when compared with the virus free control and the viral infected clone 3236 during 56, 84, 168 days of cold storage. When averaged over cold storage period, the mean berry crispiness scores were significantly higher ($P \leq 0.05$) in the viral infected clone 3236 + 3215, clone 3215

when compared with mean berry crispiness scores of the virus free control and the viral infected clone 3236. When averaged over treatments, the mean crispiness scores were significantly lower ($P \leq 0.05$) at 112, 140 and 168 days in cold storage when compared with the mean crispiness scores at 0, 28, 56 and 78 days in cold storage.

5.3.4.4. Flavour

The berry flavour decreased in the viral infected clone 3236 and virus free control during 0 to 168 days of cold storage (Table.5.17). Sensory analysis scores for flavour in the viral infected clone 3215 and clone 3236 + 3215 were significantly higher ($P \leq 0.05$) than in the virus free control from 28-168 days of cold storage. When averaged over cold storage period, the mean sensory analysis scores for flavour were higher in the viral infected clone 3215 and clone 3236 + 3215 when compared with mean flavour values of the virus free control and viral infected clone 3236. When averaged over treatments, the mean flavour scores were significantly higher ($P \leq 0.05$) following 0, 28, 56 and 84 days cold storage when compared with mean flavour values at 112, 140 and 168 days in cold storage. The interaction between treatments and cold storage period was found to be significant ($P \leq 0.05$) for berry flavour.

5.3.4.5. Overall acceptability

Sensory analysis scores for overall berry taste acceptability were found to decrease in the viral infected clone 3236 and the virus free control during cold storage (Table.5.18). Overall acceptability scores were significantly higher ($P \leq 0.05$) in the viral infected clone 3215 + 3236 and clone 3215 following 84, 112, 140 and 168 days cold storage when compared with viral infected clone 3236 and the virus free control. When averaged over cold storage, the mean overall acceptability scores were significantly higher ($P \leq 0.05$) in the viral infected clone 3236, clone 3215 + 3236, and clone 3215 than the virus free control. When averaged over treatments, the mean overall berry acceptability scores were significantly higher ($P \leq 0.05$) at 0, 28, 56 and 112 days than the values obtained after 140 and 168 days of cold storage.

Table 5.13. Changes in berry sweetness in ‘Crimson Seedless’ grapes during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	7.8	7.6	7.0a	6.9a	6.9a	6.9	6.9a	7.2a
Clone 3236	6.9	6.9	6.2b	6.2b	6.1a	5.9	4.7c	6.1c
Clone 3236 + 3215	7.1	7.1	6.6ab	6.1b	6.1b	6.6	6.1ab	6.5b
Clone 3215	6.9	6.6	6.3ab	6.2b	6.0b	5.9	5.9b	6.3bc
Mean (D)	7.2A	7.1A	6.6B	6.3B	6.3BC	6.4B	5.9C	
LSD ($P \leq 0.05$)	T = 0.4, Days = 0.5, T × Days = ns.							

ns = not significant.

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.14. Changes in sourness values of "Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	6.2	6.8	6.8a	6.8	6.8	6.8	6.8	6.7ab
Clone 3236	6.4	6.3	6.0b	6.8	6.8	6.3	6.8	6.5b
Clone 3236 + 3215	5.9	6.6	6.6ab	6.3	6.3	6.6	6.3	6.4b
Clone 3215	7.2	6.7	6.7ab	6.8	6.8	6.7	6.8	6.8a
Mean (D)	6.4	6.6	6.5	6.7	6.8	6.6	6.7	
LSD ($P \leq 0.05$)	T = 0.32, Days = ns, T × Days = ns.							

ns = not significant.

DAV = days after veraison.

Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.15. Changes in crispiness of 'Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	6.5c	6.4	6.2b	5.4b	3.7b	4.0b	3.0c	5.0c
Clone 3236	6.7bc	6.5	6.3b	6.2b	6.1a	5.7ab	4.6b	6.0b
Clone 3236 + 3215	8.3ab	8.0	7.7a	8.5a	7.5a	7.5a	7.6a	7.9a
Clone 3215	8.4a	8.2	8.1a	8.0a	7.7a	7.7a	7.7a	8.0a
Mean (D)	7.5A	7.3A	7.1A	7.3A	6.3B	6.2B	5.7B	
LSD ($P \leq 0.05$)	T = 0.6, Days = 0.7, T × Days = ns.							

ns = not significant.

DAV = days after veraison. Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.16. Changes in flavour of 'Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	6.6	5.4a	5.4c	4.6d	3.3c	3.1c	3.1c	4.5c
Clone 3236	7.0	6.7b	6.2bc	6.1c	6.1b	5.5b	4.5a	6.0c
Clone 3236 + 3215	6.6	6.8b	7.2ab	7.5b	7.2ab	7.5a	7.2a	7.1b
Clone 3215	7.4	7.4b	8.2a	8.0a	7.7a	7.6a	7.7a	7.7a
Mean (D)	6.9A	6.6A	6.6A	6.6A	6.1B	5.9B	5.6B	
LSD ($P \leq 0.05$)	T = 0.4, Days = 0.5, T × Days = 0.9.							

ns = not significant.

DAV = days after veraison. Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

Table 5.17. Changes in the overall acceptability of 'Crimson seedless' berry during cold storage period influenced by the infection of mild isolates of grapevine viruses.

Treatments (T)	Cold storage period (Days)							Mean
	0	28	56	84	112	140	168	(T)
Control (virus free)	6.7	6.0	6.4b	4.7c	5.1b	4.7b	4.4b	5.5c
Clone 3236	7.0	6.9	6.1ab	6.1bc	6.1ab	5.6b	4.4b	6.0b
Clone 3236 + 3215	6.8	7.2	7.9ab	7.1ab	7.4a	7.5a	7.4a	7.3a
Clone 3215	7.4	7.3	8.3a	7.1a	7.9a	7.6a	7.3a	7.7a
Mean (D)	7.0AB	6.9AB	7.2A	6.5ABC	6.6AB	6.4C	5.9C	
LSD ($P \leq 0.05$)	T = 0.5, Days = 0.7, T × Days = ns.							

ns = not significant

DAV = days after veraison. Means followed by the same lowercase letters within a column and means followed by the same uppercase letters within a row are not significantly different at ($P \leq 0.05$) by the least significant difference (LSD), control - virus free, clone 3236 (LRV 3 + LRV3), clone 3236 + 3215 – (LRV3 + GVA + LRV9 + LRV5) and clone 3215 (LRV3 + LRV3 + GVA + LRV9 + LRV).

5.4. Discussion

5.4.1. Berry colour

Irrespective of the viral infection, there was a gradual decrease in berry CIE L* values and h^o angle (Tables 5.2, 5.5) and an increase in CIE a* and b* values (Tables 5.3, 5.4) in all the clones during cold storage period. This decrease in berry CIE L* values, h^o angle and increase in berry CIE b*, a* values in all the clones during storage period indicates the improvement in the colour of 'Don Mariano' grape berries in cold storage attributed to moisture loss (Carreño et al., 1995). Possibly, this may be due to the increased anthocyanin accumulation at low temperature storage as reported by Maria et al. (2008) who noted darker coloured grape berries in cultivar 'Cardinal' due to an increase in anthocyanin content after 22 days of cold storage. The increase in berry C* value in the viral infected clone 3236 and clone 3236 + 3215 after 84 and 112 days of cold storage (Table 5.6), respectively may due to the decrease in CIE b* value (Table 5.4) after 84 and 112 days in cold storage. Averaged over cold storage periods, the mean CIE a* value of berries in virus free control was higher than that of the viral infected clone 3236, clone 3236 + 3215 and clone 3215 (Table 5.3). This may be attributed to the effects of grapevine leafroll virus infection on reducing colour in infected clones as reported earlier in grapevine leafroll infected 'Crimson Seedless' grape clone 314 (Jayasena and Cameron, 2008).

5.4.2. Textural properties

The decrease in berry hardness was not significant until 120 days of cold storage in the viral infected clones 3215 and 3236 + 3215 and until 56 days in the virus free control (Table 5.7). However, the decline in berry hardness was more rapid in the virus free control than in the viral infected clones 3215 and 3236 + 3215. Possibly, it may be attributed to the reduced ethylene production in the viral infected clones 3215 and 3236 + 3215 than the virus free control. Dokoozlian et al. (1995) reported earlier that exogenous application of ethephon® (426 mL per acre) at 5-10% berry colour enhanced berry colour development but reduced berry firmness in 'Crimson Seedless' grapes. Additionally, grapevine leafroll virus infection has also been reported to reduce colour development in clones 314 and 306 of 'Crimson Seedless' grapes (Brar et al., 2008). These reports signify that the ethylene hormone plays an important role in colour development and softening of 'Crimson Seedless' berries. Hence, the reduction in berry firmness in the viral infected clones 3236 + 3215 and

3215 during cold storage may be due to reduced biosynthesis of the ethylene but warrants to be investigated. There was a significant decrease ($P \leq 0.05$) in berry hardness and cohesiveness in the viral infected clone 3215 and 3236 + 3215 after 140 days in cold storage (Tables 5.7, 5.8). This may be attributed to the activity of berry softening enzymes in the berry tissues during cold storage period Takeda et al. (1983) have previously reported a decrease in berry firmness of grapes stored at low temperatures due to the degradation of pectin polymers in 'Muscadine' grapes. It may also be argued that the water loss from berry during the cold storage period may be further contributing to the above mentioned phenomena. The table grapes are prone to water loss during prolonged storage period (Kader, 1993). In contrast, the viral infected clone 3236 showed significant decrease ($P \leq 0.05$) in berry hardness after 28 days of cold storage (Table 5.7) which needs further investigation. When averaged over storage period, the mean berry hardness in the viral infected clone 3236 + 3215 and 3215 were significantly higher ($P \leq 0.05$) than the virus free control and the virus infected clone 3236 (Table 5.7). Jayasena and Cameron (2008) have earlier reported higher crispiness in sensory analysis in the virus infected clone 314 over virus free clone in 'Crimson Seedless' grapes. Apart from hardness, the other textural properties such as cohesiveness, springiness and gumminess were also higher in the virus infected clones 3236 + 3215 and 3215 than the virus free control (Tables 5.7, 5.8, 5.9, 5.10). The higher berry springiness, cohesiveness and gumminess in the viral infected clones 3236 + 3215 and 3215 may be a contributing factor for high berry hardness in these clones. It may be ascribed to infection of mild isolates of grapevine leafroll virus but requires further investigations.

5.4.3. SSC and TA

The SSC of the berry showed a significant increase ($P \leq 0.05$) after 140 days of cold storage in the viral infected clone 3236 + 3215 and after 112 days in the virus free control (Table 5.11). This may be due to decreased berry volume and concentration of sugar molecules in grape berries as a result of water loss and senescence process during storage. In 'Sultania' grapes, water loss has been found to coincide with the increased SSC during storage (Lydakis and Aked, 2003). In contrast, the grape berries from the viral infected clone 3236 showed decrease in SSC after 56 days in cold storage (Table 5.11). This may be due to senescence of the berries as reported earlier in 'Flame Seedless' grapes by Mahajan et al. (2010). They found that the

SSC increased in 'Flame Seedless' grapes until 45 days in cold storage and declined afterwards due to delayed metabolic activities and senescence of the fruit. Averaged over cold storage period, the mean SSC in the berries from virus free control was higher in comparison to the viral infected clones 3215 and clone 3236 + 3215 (Table 5.11) which may possibly due to the influence of grapevine leafroll virus infection on SSC of the viral infected clones as mentioned earlier in experiment 1 Section 4.3.3.1. The TA did not show any significant difference in the berries from the viral infected clone 3236, clone 3236 + 3215, clone 3215 and the virus free control during the cold storage (Table 5.12). This may be due to transpiration process which utilises organic acids as claimed earlier in 'Flame Seedless' grapes (Mahajan et al., 2010; Morris et al., 1992).

5.4.4. SSC: acid ratio

The SSC: acid ratio in the virus free control, viral infected clone 3236 + 3215 and clone 3215 was higher after 140 days of cold storage (Table 5.13). This may be due to increase in SCC and constant TA of the berries in these treatments after 140 days of cold storage. The mean SSC: acid ratio in the berries from viral infected clone 3236 was significantly ($P \leq 0.05$) lower when compared with the virus free control, clone 3215 and the clone 3236 + 3215. This may be due to decrease in SSC (Table 5.11) and slight increase in TA during cold storage (Table 5.12). Averaged over storage period, the mean SSC: acid ratio in the viral infected clone 3215 were lower as compared with the mean SSC: acid ratio in the virus free control which is due to lower SSC and constant TA in the juice of berries as shown in (Tables 5.11 and 5.12). Similarly, lower SSC: acid ratio has been reported from the virus infected clone 314 in 'Crimson Seedless' due to higher TA over virus free clone 5560 (Jayasena and Cameron, 2008).

5.4.5. Sensory analysis

The decrease in sweetness in the viral infected clone 3236 after 56 days of cold storage (Table 5.15) may be related to the decrease in SSC (Table 5.11). Averaged over storage period, the mean sweetness scores of berries from the viral infected clones 3236, 3236 + 3215 and 3215 were lower than the virus free control. This may be attributed to the influence of lower SSC in viral infected clones (Table 5.11). The results are in contrast to the previous report of higher sweetness scores in spite of

lower SSC in the viral infected clone 314 after one month of cold storage of ‘Crimson Seedless’ grapes (Jayasena and Cameron, 2008).

There was a significant decrease in the crispiness in all treatments including the viral infected clones 3236, 3236 + 3215 and 3215 and the virus free control during cold storage period (Table 5.16). This may be due to degradation of pectin in the cell wall and softening of berry tissues as reported in ‘Red Malaga' table grapes (Yahuaca et al., 2001). Averaged over cold storage period, the mean crispiness of berries from the viral infected clone 3236 and the virus free control were lower in comparison to the viral infected clone 3236 + 3215 and clone 3215. This may possibly due to lower berry hardness, cohesiveness, springiness and gumminess in the viral infected clone 3236 and the virus free control (Tables 5.7, 5.8, 5.9, 5.10).

The berries from the viral infected clone 3236 + 3215 and 3215 showed higher flavour scores and overall acceptability than the virus free control and viral infected clone 3236 (Tables 5.17, 5.18). The SSC: acid ratio of berries in the viral infected clone 3236 + 3215 and clone 3215 has influenced overall acceptability which is in conformity to the previous report in ‘Crimson Seedless’ (Jayasena and Cameron, 2008).

In conclusion, the viral infected clones 3236 + 3215 and 3215 did not show significant changes in SSC, TA, berry hardness, springiness, cohesiveness, and gumminess. Further, higher sensory scores for berry crispiness, flavour and over all acceptability were also observed in the viral infected clones 3236 + 3215 and 3215, until 140 days in cold storage. Hence, it can be concluded that the viral infected clone 3236 + 3215 and clone 3215 can be stored for 140 days in cold storage with acceptable colour, berry textural properties and better sensory parameters.

CHAPTER 6

General discussion, conclusion and future research

6.1. Introduction

Berry sweetness, firmness, flavours are the paramount factors in determining the consumer acceptability ratings and storage life in table grapes (Clingeleffer, 1985). 'Crimson Seedless' grapes with oblong shape and crispy berries are gaining more importance in WA. Grapevine leafroll virus infection was found to lower SSC and increase TA of berries in 'Albarino' vines (Cabaleiro et al., 1999). Similarly in 'Nebbiolo' clones (GLRaV-3 + GVA) was reported to lower total anthocyanin, SSC and increase TA. Grapevine leafroll associated virus infection has been reported to reduce berry colour development in clone 314 and clone 306 in 'Crimson Seedless' grapes (Brar et al., 2008). Contrarily in WA the grapevines inoculated with the mild strains of grapevine leafroll associated viruses has been reported to produce berries with firm flesh, in 'Crimson Seedless' clone 314 than the virus free standard clone. In general being non-climacteric habitat grapes undergoes deterioration during storage which includes stem browning, water loss, berry decay, berry softening (Crisosto et al., 2001; Perkins-Veazie et al., 1992). Hence, this research project was mainly focused on role of infection of the mild isolates of grapevine leafroll associated viruses on colour, textural properties, SSC, TA, SSC: acid ratio and sensory parameters during maturation and ripening and their influence on quality during cold storage of 'Crimson Seedless' grapes.

6.2. Effects of infection of mild isolates of grapevine leafroll viruses, grapevine viruses on the rheological properties, colour, SSC and TA during berry maturation and ripening of 'Crimson Seedless' grapes.

Grapes are profound with lot more physiological changes during ripening (Pratt, 1971). Ripening phase of grape berry includes loss of chlorophyll, accumulation of anthocyanin and berry colour development in pigmented cultivars, berry softening, accumulation of sugars and reduction in organic acids such as tartaric and malic acid (Coombe, 1992; Coombe and Bishop, 1980; Coombe and Hale, 1973; Mullins et al., 1992). There has been enormous findings reported for grapevine leafroll infection and their destructive effects on grape cultivars (Guidoni et al., 1997; Lee and Martin, 2009; Winkler et al., 1974). However the clones developed in WA with inoculation

of mild isolates of grapevine leafroll associated viruses has been reported to produce heavier berries than the virus free clones has been reported in 'Crimson Seedless' grapes (Brar et al., 2008). Therefore, the present research was focused on investigating the influence of infection of the mild isolates of GLRaV, GVA on berry rheological properties, berry colour, SSC, TA, and SSC: acid ratio in 'Crimson Seedless' grapes during maturation and ripening.

Grapevines inoculated with the mild isolates of (GLRaV, GVA) in 'Crimson Seedless' grapes clone 3236, clone 3236 + 3215, clone 3215 and the virus free control were used in this experiment. Improvement in colour of the berries were noted in all clones during maturation and ripening which confirms the earlier reports (Coombe, 1992) that colour development in pigmented cultivars was due to the accumulation of anthocyanin accumulation at stage III of grape berry growth. Berries from the viral infected clone 3236, clone 3236 + 3215 and clone 3215 showed reduced colour development than berries from the virus free control. Similarly, in 'Crimson Seedless' grapes virus infected clone 314 and 306 has been reported with reduced berry colour development than the virus free clones, may possibly be due to lower levels of Cn3glc, Dp3glc, Pt3glc, Pn3glc and Mv3glc anthocyanins (Brar et al., 2008). Reduction in accumulation of anthocyanin may be due to lower activity of the enzymes involved in biosynthesis of anthocyanins as reported in 'Nebbiolo' grapes (Guidoni et al., 1997). It may also be claimed that reduction in berry colour of the viral infected clone 3236, clone 3236 + 3215 and clone 3215 may be due to reduced photosynthates supply to grape berries (Gholami, 2004) as reported earlier in 'Albarino' vines and 'Nebbiolo Clone' that grapevine leafroll virus infection reduce photosynthesis in leaves (Cabaleiro et al., 1999; Guidoni et al., 1997). Berry hardness was decreased during maturation and ripening in all the clones irrespective of the virus infection which may possibly attributed to the increased activity of softening enzymes during the ripening phase of grape berry (Coombe, 1960; Coombe and Hale, 1973). When averaged over ripening time, the mean berry hardness was higher in the viral infected clones 3236 + 3215 and 3215 as compared with virus free control and it was consistent with previous report that the virus infected clone 314 which showed higher crispiness scores in sensory analysis than the virus free standard clone (Jayasena and Cameron, 2008). Berries from virus free control has lower hardness which may be claimed due to the high SSC in berries during

maturation and ripening as similar to previous report by (Lee and Bourne, 1980) in 'Barbera' grapes he found a negative correlation between SSC and berry firmness. Berry springiness, cohesiveness, gumminess were higher in the viral infected clones 3236 + 3215 and 3215 than the virus free control and this may be a contributing factor for higher berry hardness in these virus infected clones. SSC of the berries in all the clones irrespective of the virus infection showed a gradual increase during maturation and ripening which were consistent with earlier findings (Coombe, 1989). The SSC of berries in 'Crimson Seedless' the viral infected clones 3236, 3236 + 3215 and 3215 were lower than the virus free control during maturation and ripening and this may possibly be due to reduced photosynthates supply in virus infected clones as reported earlier (Cabaleiro et al., 1999; Guidoni et al., 1997). There was a decrease in TA of berries in all clones irrespective of virus infection similar to earlier reports (Kluba et al., 1978) and this may possibly be due to decrease in malic acid during ripening phase (Hardy, 1968). Higher TA was recorded in the virus infected clones than the virus free control may possibly be due to high levels of malic acid and lower levels of tartaric acid as reported in 'Crimson Seedless' grapes (Brar et al., 2008). The SSC: acid ratio was slightly lower in the viral infected clones 3236, 3236 + 3215 and 3215 than those observed in the berries from the virus free control during maturation and ripening. This may be attributed to higher TA in all the viral infected clones as has been reported previously in 'Crimson Seedless' grapes (Jayasena and Cameron, 2008).

6.3. Influence of infection of mild isolates of grapevine viruses on cold storage life and quality in 'Crimson Seedless' grapes.

The influence of post-harvest storage techniques on quality of grapes had been reported in various cultivar such as 'Sultania' (Lydakis and Aked, 2003). Influence of grapevine leafroll associated viruses on quality of grapes had been reported in 'Crimson Seedless' grapes as it reduced anthocyanin accumulation, without influencing SSC, TA of the virus infected berries. Sensory analysis in 'Crimson Seedless' grapes from the virus infected clone 314 after one month of storage had been reported to produce crispier berries than the virus free standard clone (Jayasena and Cameron, 2008). The aim of this present research was to investigate the effects of GLRaV, GVA on storage life and quality of 'Crimson Seedless' grapes in cold storage.

Grapevines inoculated with mild isolates of (GLRaV, GVA) in 'Crimson Seedless' grapes clone 3236, clone 3236 + 3215, clone 3215 and the virus free control were used in this experiment for cold storage period of 168 days. The results showed that berries from all clones irrespective of virus infection showed a gradual decrease in L*, h^o, b* values and increase in a* values in cold storage period which indicates the colour development in berries as shown earlier in 'Don Mariano' cultivar (Carreño et al., 1995). This colour development in cold storage period may be attributed to increased accumulation of anthocyanin as reported earlier in 'Cardinal' grapes where increase in anthocyanin accumulation was noted during 22 days of cold storage (Maria et al., 2008). Averaged over cold storage period the mean a* values were higher in the virus free control which showed higher colour than the viral infected clone 3236, clone 3236 + 3215, clone 3215 and this may be attributed to viral infection as reported earlier in 'Crimson Seedless' grapes. These grapes showed lower colour in the clone 314 than the virus free standard clone (Jayasena and Cameron, 2008). The berry hardness was significantly higher in the viral infected clone 3236 + 3215, clone 3215 than the virus free control during cold storage period and this may possibly be due to decrease in biosynthesis of ethylene in the viral infected clones. The previous report in 'Crimson Seedless' showed that exogenous application of ethephon® (426 mL per acre) at 5-10% colour break has enhanced berry colour and reduced berry firmness (Dokoozlian et al., 1995). The infection of grapevine leafroll virus has been reported to reduce berry colour development in 'Crimson Seedless' clones 314 and 306 (Brar et al., 2008). These earlier reports highlights the role of ethylene in berry colour development and berry softening and hence it can be argued that delay in loss of berry firmness in the virus infected clones 3236 + 3215 and 3215 may possibly be due to reduction in biosynthesis of ethylene and needs to be investigated further. Averaged over storage period, the mean berry hardness were higher in the viral infected clones 3236 + 3215 and 3215 as compared with the virus free control which has been reported earlier in 'Crimson Seedless' grapes clone 314 which showed higher crispiness than the virus free standard clone (Jayasena and Cameron, 2008). Berry cohesiveness, springiness, and gumminess in the viral infected clone 3236 + 3215, clone 3215 were higher than the virus free control during cold storage which may be a contributing factor for higher berry hardness in the viral infected clone 3236 + 3215 and clone 3215. The SSC in the

viral infected clone 3236, clone 3236 + 3215 and clone 3215 were higher than the virus free control which may possibly be due to influence of grapevine leafroll associated virus on the virus infected clone as mentioned earlier in experiment 1 Section 4.3.3.1. TA remains unchanged during cold storage period in all the clones and this may be due to the utilization of organic acids during transpiration process as claimed earlier in 'Flame Seedless' grapes (Mahajan et al., 2010). Averaged over storage period, the mean SSC: acid ratio in the viral infected clone 3215 were lower than the virus free control which may possibly be due to lower SSC and constant TA levels in the virus infected clone 3215. Similarly 'Crimson Seedless' clone 314 has been suggested to have higher SSC: acid ratio due to the influence of higher TA than the virus free standard clone (Jayasena and Cameron, 2008).

Sweetness scores in the virus free control was higher during cold storage period as compared with the virus infected clones which are in contrast to previous report where 'Crimson Seedless' clone 314 was rated with higher sweetness scores than the virus free standard clone (Jayasena and Cameron, 2008). Over all the sensory scores such as berry crispiness, berry flavour, overall acceptability score ratings were higher during cold storage in the viral infected clone 3236 + 3215 and clone 3215 than the virus free control. Higher crispiness scores in the virus infected clone may be due to the higher textural properties such as berry hardness, gumminess, cohesiveness, springiness (Table 5.6, 5.7, 5.8, 5.9). These results are consistent with previous findings (Jayasena and Cameron, 2008) where clone 314 was reported to score higher for crispiness, over all acceptability and flavour.

6.4. Conclusion

Influence of infection of mild isolates of grapevine leafroll associated viruses and grapevine viruses on 'Crmison Seedless' berry colour, textural properties, SSC, TA and SSC: acid ratio during maturation and ripening has been studied.

1. The infection of mild isolates of the grapevine leafroll associated virus and grapevine virus reduced berry colour development in the viral infected clone 3236, clone 3236 + 3215 and clone 3215 compared to the berries from the virus free control vines.
2. The SSC in berries of the virus infected clones were lower than the virus free control.

3. Infection of mild isolates of grapevine leafroll associated viruses was found to enhance berry cohesiveness, springiness in the viral infected clones 3236 + 3215 and 3215 than the virus free control.
4. Infection of mild isolates of grapevine leafroll associated virus does not influence TA.

Influence of mild isolates infection of grapevine viruses on cold storage life and quality in 'Crimson Seedless' grapes.

1. Mild isolates of grapevine leafroll associated virus along with grapevine infection maintained berry hardness, springiness, cohesiveness and gumminess in viral infected clones 3236 + 3215 and clone 3215 during cold storage period.
2. Virus infected clones 3236 + 3215 and clone 3215 can be stored at $0 \pm 0.5^{o}C$ in cold storage for period of 140 days with acceptable colour, higher sensory scores such as (berry crispiness, berry flavour and over all acceptability).

6.5. Future research

The present research provides the information on the influence of infection of the mild isolates of GLRaV and GVA on berry colour, textural properties such as berry hardness, springiness, gumminess, cohesiveness, SSC, TA and SSC: acid ratio in 'Crimson Seedless' grapes during maturation and ripening. Their influence on quality and sensory parameters during their post-harvest cold storage period has also been discussed.

1. Berry textural properties such as berry cohesiveness, gumminess and springiness were higher in the virus infected clones 3236 + 3215 and 3215 in 'Crimson Seedless' grapes during maturation and ripening. We did not determine the actual mechanism involved in berry softening during this period. Therefore the future research should focus on elucidating the role of grapevine leafroll associated viruses on enzymes involved in the softening of these berries.
2. Virus infected clone 3236 + 3215 and clone 3215 in 'Crimson Seedless' grapes exhibited higher berry hardness, gumminess, springiness and cohesiveness during cold storage period and there was a delay in loss of firmness which may possibly be attributed to reduction in biosynthesis of

ethylene which was not measured and warrants investigation on biosynthesis of ethylene during cold storage in the berries from virus infected vines.

3. However, the viral infected clone 3236 which showed a significant deviation in SSC and textural properties from other the virus infected clones 3236 + 3215 and 3215 which may be due to the infection of different mixture of viruses but needs further investigation.

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