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RESEARCH ARTICLE

SEASONAL VARIATION IN LEAF WATER, LIPID CONTENT AND THE CORRESPONDING COLD ACCLIMATION IN SUBTROPICAL FRUIT PLANTS

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ABSTRACT

Frost is one of the most serious threats to the subtropical horticulture in the Western plains and lower Himalayan region. Modulations in leaf water and lipid content and their linkage to low temperature acclimation process have been worked out in twenty five subtropical fruit plant species through relative electrolyte leakage (REL) studies. Acclimation values of different species were calculated on the basis of REL (%) observed under open field and polyhouse grown plants. Mechanism of frost stress tolerance in different species has also been highlighted through these studies. The species studied have been found fall under six distinct categories as far as modulation in leaf water, lipid and acclimation level acquired (adjudged through the decrease in relative electrolyte leakage upon freezing exposure) was concerned. In many of the species studied this mechanism has been explained but some of the species still need further investigations for verification of the processes associated with the acquisition tolerance to the low temperature stress in these plants.

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INTRODUCTION

Frost induced freezing is a major environmental stress in the subtropics which cause serious economic damage to crops and limit their distribution across the geographical boundaries. Major damage to the subtropical fruit species is imparted by the excessive cooling of plant surfaces and subsequent freezing of inter or intra-cellular water. As the plants are immobile, they are bound to bear the stress under natural conditions and the extent of damage experienced by them is mainly dependent on the fact that how fast the temperature drops, the level of acclimation attained by the plant species and thereby their ability to super cool before freezing. To withstand freezing, cold hardy plants possess mechanisms to regulate formulation of ice in their tissues. But, the subtropical plants lack this intrinsic defense mechanism against frost or low temperature stress. Even if all aspects of crop production are well managed, one night of subzero temperatures may lead to complete crop loss in many of these species. Freezing injury in plants is primarily a consequence of membrane destabilization resulting from freeze induced dehydration (Steponkus, 1984). Low temperature tolerance of the plants requires an orchestration of different plant processes that occur during acclimation and which ultimately contribute to the increased stability of cellular membranes during freeze induced dehydration. The stability of the cellular membranes during freezing is further depends upon number of functional and structural characteristics of the plasma membrane. The fundamental structure of plasma membrane is the phospholipid bilayer which form stable barrier between the aqueous compartments of the cell. Recent studies suggest that lipids are the fundamental structural unit of the plasma membrane and are largely responsible for the membrane integrity under the stress conditions (Cooper, 2000) and have special significance as they are directly associated with membrane plasticity and freezing tolerance of the plants. But, as stated above the freezing process is associated with dehydration, the freezing injury caused thus must be having linkage with the water status of the plant and organ suffering freezing. Thus, the plant species which acclimate to low temperature stress, try to manipulate their lipid and/or water status so as to increase freeze avoidance, even if only by one or a few degree Celsius. Keeping in to consideration the facts stated above, efforts were made to quantify the association of leaf water and lipid content with their frost induced freeze damage observed in different subtropical fruit species so as to speculate the optimized protection measure against the frost.

MATERIALS AND METHODS

The studies were conducted at the College of Horticulture and Forestry, Dr YS Parmar University, Neri, Hamirpur (HP) India.

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For the studies twenty five (25) subtropical fruit species namely: 1.*Aegel marmelos* (Bil) 2.*Annona squamosa* (Custard Apple) 3.*Artocarpus heterophylus* (Jack Fruit) 4.*Artocarpus lakucha* (Dheu) 5.*Carica papaya* (Papaya) 6.*Carissa carandus* (Karonda), 7.*Citrus aurantifolia* (Lime) 8.*Citrus pseudolimon* (Galgal) 9.*Citrus reticulata* (Mandarin) 10. *Citrus sinensis* (Sweet orange) 11.*Cordia myxa* (Lasura) 12. *Emblica officinalis* (Aonla) 13.*Eriobotrya japonica* (Loquat) 14.*Ficus carica* (Fig) 15.*Fragaria ananasa* (Strawberry) 16.*Grewia asiatica* (Phalsa) 17.*Litchi chinensis* (Litchi) 18.*Mangifera indica* (Mango) 19.*Musa paradisiaca*(Banana) 20.*Passiflora edulis* (Passion Fruit) 21.*Psidium guajava* (Guava) 22.*Punica granatum* (Pomegranate) 23.*Rubus ellipticus* (Aakhe) 24.*Syzigium cumunii* (Jamun) and 25.*Ziziphus mauritiana* (Ber) were selected. The container raised plants of these species were grown under polyhouse conditions as well as under open field conditions. In order to provide natural exposure to decreasing temperature from autumn to winter one lot of containerized plants of each species was grown under open field conditions whereas one lot of plants was grown under polyhouse conditions at 30^0C so that these could not get the necessary cue of decreasing temperature from autumn to winter. The open field grown plants were studied for the changes in the leaf water and lipid content from October 15 to December 15 at fortnightly interval. The leaf water content was measured as percent of water lost per unit of fresh leaf weight after oven drying of leaves at 65^0C for 5 days. The dried mass of the same leaves was taken for the total lipid estimation through Soxhlet extraction as described by Shahidi (2001). The content of total lipid estimated was expressed as mg per g of leaf dry weight for each of the species studied.

In order to adjudge the acclimation potential and the corresponding frost tolerance of different fruit species the open field grown and polyhouse grown containerized plants were subjected to frost stress at -2^0C for four hours in a controlled frost simulation chamber. The frost induced freeze damage was estimated through Relative Electrolyte leakage (REL%) studies as described by Sharma (2012) and Soliemani (2003). Periodic assessment of REL was made for ascertaining the acquired acclimation to frost and low temperature stress. The REL differences of controlled environment (polyhouse) plants - R_C and uncontrolled environment (Open Field) plants - R_{UC} have been used for quantification of the genetic potential of a particular species to acclimatize itself to frost and low temperature stress. The acclimation value (A_{VC}) for each species was calculated as: $A_{VC}=\{(R_C-R_{UC})/R_C\}x100$. The studies were carried out for two years and the data were pooled and analyzed statistically as per procedure described by Gupta and Gupta (1995). The mathematical tool 'Venn diagram' (Anon., 2017) has been used for grouping different fruit species as per modulation of leaf lipid and water content and the seasonal acclimation acquired. The data on environmental variables like temperature and humidity were recorded with the help of Madgetech (RHTemp.101) automatic data logger.

RESULTS AND DISCUSSION

Seasonal Variation in leaf lipid and water content

The data presented in Table 1 reveal that the leaf lipid content did not varied significantly with the onset and progression of winters in many of the species studied. But, there were some species which recorded significant increase in the total lipid content of the leaf samples, though none of the species studied exhibited decrease in the lipid content. The average lipid content in different species studied varied from 6.4mg/g to 21.5mg/g. The lowest lipid content was recorded for *Ficus carica* followed by *Artocarpus lakucha, Musa paradisiaca, Cordia myxa and Carica papaya*. The highest lipid content was recorded in case of *Citrus* spp., *Punica granatum, Aegel marmelos, Rubus ellipticus and Fragaria ananasa.* The highest seasonal increase in leaf lipid content was observed in case of *Citrus* species which exhibited 2 to 3mg/g increase in total lipid content. Apart from this, the other species like *Rubus elipticus* and *Ziziphus mauritiana* also observed around 2 mg/g increase in the total lipid content. The species like *Emblica officinalis, Litchi chinensis* and *Punica granatum* also exhibited significant increase in lipid content which indicated that these have significantly responded significantly to the low temperature environmental cue and have tried to manage their physiological functions in view of the onset of low temperature stress. These findings are in conformity with those of Kramer and Kozlowsk (1979) who have reported that some plant species accumulate lipids with the onset of winters.

Leaf water content also varied significantly in some of the fruit species studied. The leaf water content varied from 48.8% (*Citrus pseudolimon*) to 84.9% (*Musa paradisiaca*). In all the specie studied it decreased but increased in none of the species. The highest decrease in leaf water content was observed in case of deciduous fruit speices like *Aegel marmelos (26%), Cordia myxa (20%), Grewia asiatica (21%)* and this decrease in water content was observed significant upto November first week and thereafter marginal decrease has been observed. The other species which have shown significant reduction in leaf water content were *Carissa carandus, Psidium guajava, Citrus pseudolimon, Emblica officinalis, Rubus ellipticus and Ziziphus mauritiana;* in these species the significant decrease has been observed upto first week of December or even upto mid of December also. Robert et al. (1980) also reported that leaf water content varies between the species and varied during the season with in the species. They demonstrated that all deciduous species show large osmotic fractions early during the season but considerable decrease as the plants advanced toward the autumn. Many of the plant species like *Anona, Artocarpus, Carica, Citrus aurantifolia, C. reticulata, C. sinensis, Frageria, Litchi, Mangifera, Musa, Passiflora, Punica, Syzigium* did not show any significant modification in leaf water content with the onset of winters. This indicates that evergreen species have differential behavior in respect of the modification in leaf water content with the onset of winters.

The data pertaining to the REL (%) recorded after the controlled exposure of the open field and polyhouse grown plants are presented in Table 2. It is evident from the data that when the plants growing under polyhouse conditions were subjected to frost stress, there was not observed any significant difference in the REL(%) recorded for a fruit species at different intervals of time from October to mid of December. It implies that there was observed almost same level REL (%) due to frost induced freeze damage with in a specie at different observation intervals. Whereas, for most of the open field grown plant species there were observed significant differences in REL values with in a species at different intervals of time. In some of the species there was observed decrease in REL value whereas in some of the species significant variation was not observed with respect of time intervals of observation during the onset of winters.

Table 1. Seasonal variation in leaf lipid and water content in different subtropical fruit species

S. No.	Fruit Plant Species	Lipid Content (mg/g)							Water Content (%)						
		15 Oct	1 Nov	15 Nov	1 Dec	15 Dec	Mean	CD (0.05)	15 Oct	1 Nov	15 Nov	1 Dec	15 Dec	Mean	CD (0.05)
1	*Aegel marmelos*	16.5	17.2	17.2	17.7	-	17.2	NS	68.4	51.3	48.2	42.3	-	52.6	12.36
2	*Annona squamosa*	11.4	11.4	11.4	11.3	11.5	11.4	NS	65.0	62.1	60.2	60.1	60.1	61.5	NS
3	*Artocarpus heterophylus*	10.6	10.6	10.9	11.2	11.2	10.9	NS	68.1	65.3	66.2	64.2	62.2	65.2	NS
4	*Artocarpus lakucha*	6.5	6.9	7.3	7.6	7.8	7.22	NS	62.0	63.2	61.8	60.5	59.2	61.3	NS
5	*Carica papaya*	8.2	8.9	8.5	8.3	8.7	8.52	NS	74.4	72.2	71.6	71.2	70.1	71.9	NS
6	*Carissa carandus*	11.7	11.7	11.8	12.2	12.9	12.1	NS	62.6	59.8	57.2	54.1	51.2	57.0	6.57
7	*Citrus aurantifolia*	18.6	20.1	20.2	20.1	20.6	19.9	1.22	65.6	62.3	62.4	61.2	61.2	62.5	NS
8	*Citrus pseudolimon*	18.6	18.6	19.9	21	22.1	20.0	1.47	54.2	49.2	48.4	47.2	45.1	48.8	1.12
9	*Citrus reticulata*	18.3	20.2	21.1	20.6	20.6	20.2	1.24	64.4	63.2	63.1	62.8	61.2	63.0	NS
10	*Citrus sinensis*	18.2	20.5	21.4	21.4	22.8	21.5	1.11	68.2	68.2	67.3	66.2	66.1	67.2	NS
11	*Cordia myxa*	8.2	8.2	8.4	8.4	-	8.3	NS	63.2	52.3	50.1	43.6	-	52.3	6.17
12	*Emblica officinalis*	14.5	14.8	15.2	15.4	16.3	15.4	0.78	61.2	59.8	57.1	55.4	52.2	57.1	2.12
13	*Eriobotrya japonica*	11.2	11.2	11.4	11.3	11.6	11.3	NS	53.2	51.2	51.1	50.3	50.2	51.2	0.78
14	*Ficus carica*	6.4	6.5	6.4	-	-	6.4	NS	63.2	58.7	51.7	-	-	57.9	5.89
15	*Fragaria ananasa*	15.7	15.6	15.8	15.9	16.1	15.9	NS	77.6	75.2	71.4	72.4	70.2	73.4	NS
16	*Grewia asiatica*	12.6	13.0	12.8	12.6	-	12.8	NS	75.2	67.2	61.1	54.2	-	64.4	7.14
17	*Litchi chinensis*	10.4	10.6	10.9	11.7	11.9	11.1	1.03	58.2	56.2	57.1	56.2	55.1	56.6	NS
18	*Mangifera indica*	15.1	15.5	15.6	15.8	15.8	15.6	NS	53.9	53.1	52.8	52.1	52.0	52.8	NS
19	*Musa paradisiaca*	7.1	7.5	7.4	7.4	7.6	7.4	NS	85.9	85.7	85.2	84.1	83.4	84.9	NS
20	*Passiflora edulis*	11.8	11.8	11.8	11.9	12.1	11.9	NS	78.7	77.4	75.2	76.2	76.5	76.8	NS
21	*Psidium guajava*	12.2	12.3	12.5	12.5	12.6	12.4	NS	61.8	59.2	57.4	52.2	53.1	56.4	1.58
22	*Punica granatum*	17.1	16.9	17.1	17.9	18.3	17.6	1.77	53.2	53.1	52.8	82.6	51.1	54.9	NS
23	*Rubus ellipticus*	16.1	16.3	16.9	17.4	18.1	17.1	1.56	63.7	62.1	58.2	57.1	56.1	59.8	1.22
24	*Syzigium cumunii*	12	12.2	12.4	12.1	12.2	12.2	NS	62.6	61.2	61.3	60.4	61.0	58.9	NS
25	*Ziziphus mauritiana*	12.2	12.4	12.6	13.4	14.2	13.1	1.83	65.1	61.2	58.1	57.2	55.4	59.4	1.47

Table 2. Frost/ low temperature stress mediated Relative Electrolyte Leakage (REL%) and acclimation value for different subtropical fruit species

S. No.	Fruit Plant Species	REL(%)														Accli-mation Value (A_{VC})
		Open Field Grown Plants							Poly House Grown Plants							
		15 Oct	1 Nov	15 Nov	1 Dec	15 Dec	Mean (A_{UC})	CD (0.05)	15 Oct	1 Nov	15 Nov	1 Dec	15 Dec	Mean (A_C)	CD (0.05)	
1	*Aegel marmelos*	21.2	18.2	15.4	15.0	-	17.0	2.11	22.6	20.2	23.1	18.2	-	20.8	NS	18.3
2	*Annona squamosa*	94.2	90.3	86.9	91.4	74.2	88.3	NS	96.5	91.2	80.2	91.2	80.2	92.1	NS	4.13
3	*Artocarpus heterophylus*	96.4	96.5	91.2	82.1	82.1	88.1	NS	91.2	94.2	90.3	92.5	91.4	95	NS	7.26
4	*Artocarpus lakucha*	86.4	86.5	91.2	82.1	82.1	86.4	NS	86.2	84.2	90.3	90.5	81.4	94.1	NS	8.18
5	*Carica papaya*	100	100	100	100	97.2	99.5	NS	100	100	100	100	100	100	NS	0.5
6	*Carissa carandus*	72.4	70.2	65.2	51.3	50.3	61.3	14.1	86.2	82.4	75.4	71.6	72.4	77.4	NS	20.8
7	*Citrus aurantifolia*	57.4	51.2	48.4	42.2	42.1	47.6	6.12	56.3	58.2	61.4	58.3	56.3	57.1	NS	16.64
8	*Citrus pseudolimon*	55.4	51.0	48.1	43.1	41.9	47.4	7.11	55.3	54.2	54.4	53.3	52.3	53.7	NS	11.7
9	*Citrus reticulata*	57.4	51.2	48.4	42.2	42.1	47.6	6.12	56.3	58.2	61.4	58.3	56.3	57.1	NS	16.64
10	*Citrus sinensis*	55.1	52.2	50.4	40.2	40.1	47.4	7.11	57.1	59.2	60.4	57.9	55.1	56.8	NS	16.55
11	*Cordia myxa*	83.4	74.2	73.5	-	-	77.0	5.63	84.2	90.3	86.9	-	-	87.1	NS	11.60
12	*Emblica officinalis*	78.4	70.2	65.2	60.3	60.3	66.1	8.17	78.2	76.2	72.4	76.3	71.6	73.9	NS	10.6
13	*Eriobotrya japonica*	14.2	13.2	14.6	13.5	13.2	13.7	NS	15.2	15.4	15.3	15.1	15.0	15.2	NS	5.56
14	*Ficus carica*	43.4	34.2	33.5	-	-	37.0	9.68	42.7	39.9	41.2	-	-	41.3	NS	10.41
15	*Fragaria ananasa*	16.1	16.8	16.2	13.7	14.2	15.4	NS	16.8	16.2	16.4	15.4	16.1	16.2	NS	4.93
16	*Grewia asiatica*	57.1	44.2	43.5	-	-	46.5	12.3	56.3	58.2	51.4	-	-	54.6	NS	14.83
17	*Litchi chinensis*	92.6	81.2	69.2	56.4	58.2	70.5	12.8	92.3	85.6	82.3	82.99	82.2	84.4	NS	16.47
18	*Mangifera indica*	95.8	80.2	75.2	67.3	66.3	75.5	NS	94.5	96.2	92.4	96.3	91.6	91.9	NS	14.85
19	*Musa paradisiaca*	95.2	96.3	95.2	91.4	96.5	95.2	NS	91.4	96.5	96.3	95.2	95.2	96.5	NS	1.35
20	*Passiflora edulis*	88.2	86.2	82.4	72.1	72.6	80.6	NS	85.4	86.3	81.6	80.2	85.2	83.5	NS	3.47
21	*Psidium guajava*	56.3	51.2	46.3	41.2	41.1	46.4	7.11	56.7	59.2	57.4	58.4	56.2	58.2	NS	20.27
22	*Punica granatum*	46.3	41.2	36.3	33.2	31.1	36.6	11.7	46.3	41.2	46.3	44.2	41.1	43.4	NS	15.67
23	*Rubus ellipticus*	12.4	11.6	10.4	10.2	10.4	11.0	NS	12.3	11.6	14.5	13.2	11.2	12.6	NS	6.28
24	*Syzigium cumunii*	61.2	56.3	51.9	51.2	50.3	53.7	4.33	62.3	64.2	61.4	61.4	64.5	62.9	NS	14.63
25	*Ziziphus mauritiana*	26.4	27.9	22.2	17.2	17.4	21.6	2.18	27.4	22.9	23.4	25.3	26.6	25.1	NS	13.94

The REL(%) decreased significantly in case of *Aegel marmelos* (Bil), *Carissa carandus* (Karonda), *Citrus aurantifolia* (Lime), *Citrus pseudolimon* (Galgal), *Citrus reticulata* (Mandarin), *Citrus sinensis* (Sweet orange), *Cordia myxa* (Lasura), *Emblica officinalis* (Aonla), *Ficus carica* (Fig), *Grewia asiatica* (Phalsa), *Litchi chinensis* (Litchi), *Mangifera indica* (Manog), *Psidium guajava* (Guava), *Punica granatum* (Pomegranate), *Syzigium cumunii* (Jamun) and *Ziziphus mauritiana* (Ber) whereas, in case of *Annona squamosa* (Custard Apple), *Artocarpus heterophylus* (Jack Fruit), *Artocarpus lakucha* (Dheu), *Carica papaya* (Papaya), *Eriobotrya japonica* (Loquat), *Fragaria ananasa* (Strawberry), *Musa paradisiaca* (Banana), *Passiflora edulis* (Passion Fruit), *Rubus ellipticus* (Aakhe), the REL(%) was almost similar at different time intervals.

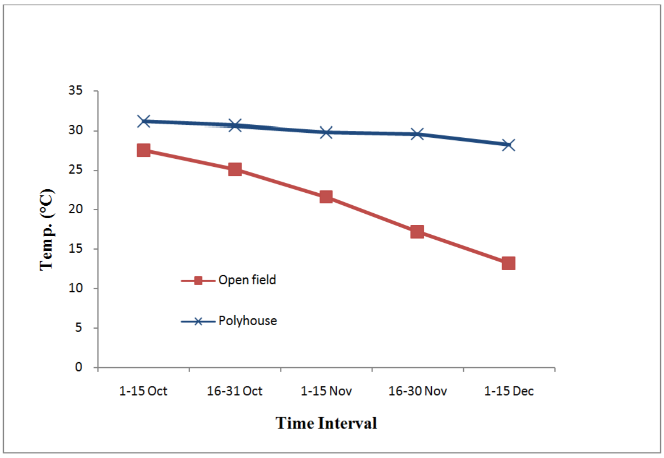

Fig. 1. Seasonal Variation in Temperature under open field and polyhouse conditions

The non significant variation in the REL (%) of the polyhouse grown plants may be attributed to the reason that under these conditions the seasonal drop in temperature was observed to be non significant (Fig. 1) thereby the continued their growth without experiencing the seasonal changes in the physiological functions of the plants. Therefore, when a plant species growing under polyhouse conditions was subjected to frost stress at different time intervals, it suffered almost similar level of freeze damage thereby resulting in non significant variation in REL values. Contrary to it the open field grown plants experienced a gradual decrease in environmental temperature (Fig. 1) which served as a environmental cue for the plants to modify their physiology in order to acclimate against the frost or low temperature stress thereby these species suffered different level of damage and thereby these species have shown different values of REL for different times of observation. This indicates that plants which have shown significant decrease in REL(%) have acclimatized to certain extent with the decreasing temperature autumn to winter. Whereas, the plants which have not shown significant decrease in REL(%) value were not able to acclimatize well with the onset of winters. This is further evidenced through the Acclimation value calculated for these species. The plants which have decreasing trend of REL(%) have been found to have higher acclimation value (Table 2). The acclimation value for different species varied between 0.5 to 20.8. The highest acclimation value has been recorded for *Carissa carandus* followed by *Psidium guajava, Aegeal marmelos, Citrus spp., Litchi chinensis, Punica granatum.* The higher values of acclimation in case of deciduous species like *Aegel marmelos* (bil), *Ficus carica* (fig), *Cordia myxa* (lasura), *Grewia asiatica* (phalsa) may be attributed to the mobilization of nutrients and other bio-molecules from the leaves prior to programmed leaf fall. The lowest acclimation value has been recorded for *Carica papaya* followed by Musa paradisiaca, *Passiflora edulis, Anona squamosa, Artocarpus heterophylus.* Other species studied have been found to have the acclimation value between these species. These findings are in conformity with those of Sharma (2008) who under a field experiment have reported almost similar type of pattern of relative frost susceptibility of some of the fruit species. Thus, from these studies it can be inferred that in some of the evergreen plant species like *Psidium guajava* (guava), *Mangifera indica* (mango), *Litchi chinensis* (litchi), *Artocarpus heterophylus* (jack fruit), *Emblica officinalis* (aonla) etc. the process of acclimation imparted certain level of tolerance against frost and low temperature stress and every such species has a threshold level of tolerance beyond which if the stress exceeds the damage has been observed. There has been found to be an exception in case of *Eriobotrya japonica, Fragaria ananasa and Rubus elipticus*, these species suffered least frost and low temperature damage but these species have also been found to have low acclimation value. Also, in case of these species there has been recorded no significant difference in the REL values of open field conditions as well as that recorded for the polyhouse grown plants. All these species belong to family rosaceae, it implies that the species belonging to family rosaceae might be having certain other intrinsic mechanism to tolerate the frost and low temperature stress which is yet to be established. Therefore it can be inferred from these studies that acclimation impart only certain level of tolerance to some of the plant species. But, apart from acclimation there may be certain other intrinsic factors which govern the frost or low temperature stress tolerance in plant species. Wisniewski and Fuller (1999) reported that in some of the species intrinsic plant anti freeze proteins probably control rather than prevent freezing by super cooling substantially and there may be certain structural features involved in this preventive mechanism.

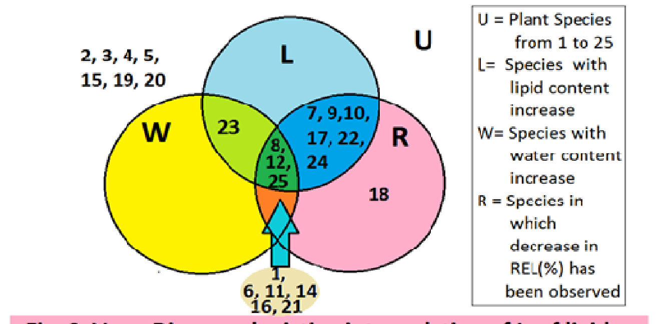

Fig. 2. Venn Diagram depicting inter-relation of Leaf lipid and water content with Relative Electrolyte Leakage (REL%)

On the basis of above discussed results it has been inferred that subtropical fruit plant species undergo various type of physiological modulations as an effort to develop certain acclimation to frost and low temperature stress. The inter-relation of the species leaf water and lipid content with the frost damage that is electrolyte leakage (REL%) has been elaborated through Venn diagram and presented in Fig 2. There were observed certain species which upon exposure to low temperature showed significant increase their lipid content, there were some which reduced their water content in some both these modulations were observed. With these modulations, there were species which developed certain level of acclimation which was reflected by the decrease in relative electrolyte leakage. On the basis of data presented in Fig. 2 regarding modulation of water and lipid content and changes in REL% the studied species were grouped into following six categories: a) From the Venn diagram it can be observed that plant species number 7, 9, 10, 17, 22, 24 i.e. *Citrus aurantifolia, Citrus reticulata, Citrus sinensis, Litchi chinensis, Punica granatum, Syzigium cumunii* have shown increase in leaf lipid content and decrease in REL% which reflects that these fruit tree species acquired certain level of acclimation by modulating lipid content. Senser and Beck (1982) have shown that lipid content of spinach do not change whereas that of Ivy and spruce do change with the onset of winters. On the other hand Chapman *et al.* (1983) has revealed that lipid class condition do not change but degree of unsaturation do change in pea leaves in winters. Therefore these studies also support the present findings that some species modulate their lipid content in a response to low temperature signal so as to develop tolerance against the ensuing freezing stress. b) Plant species number 6, 11, 14, 16, 21 i.e. *Aegel marmelos, Carissa carandus, Cordia myxa, Ficus carica, Grewia asiatica, Psidium guajava* have modulated their water content and have also shown decrease in REL. But, here in this case most of these species are deciduous except *Carissa* and *Psidium* spp., so the decrease in water content may be due to the onset of senescence in deciduous species, but in case of *Carissa* and *Psidium* the decrease in water content and subsequent decrease in REL may be attributed as a resultant of acclimation process. These findings are in conformity with those of Tyree and Jarvis (1982) who reported that large changes in water content occurs during onset of winters especially in temperate plants (mostly deciduous) and it appears to be correlated to the changes in frost tolerance. They further added that there are some species which show little or no adjustment in their leaf water content. c) Similarly the third category was observed to be of fruit plant species which have increased their lipid content as well as reduced their leaf water content; plant species no. 8, 12, 23 and 25 i.e. *Citrus pseudolimon, Emblica officinalis, Rubus ellipticus, Ziziphus mauritiana.* All these species except Rubus ellipticus have shown decreased REL%, means these species have acclimated to the low temperature stress but the same phenomenon has not been observed in case of *Rubus ellipticus* which otherwise possesses high level of tolerance to low temperature stress therefore this species has been put into another category *i.e.* d) the species which undergo modulation of water and lipid content but do not undergo acclimation and survive low temperature stress due to some other intrinsic mechanism. e) The fifth group of species observed was one which neither modulate water content nor lipid content and suffer heavy frost damage and these species were: 2, 3, 4, 5, 15, 19, 20 i.e. *Annona squamosa, Artocarpus heterophylus, Artocarpus lakucha, Carica papaya, Mangifera indica, Musa paradisiaca, Passiflora edulis*. f) The sixth category was observed that of species no. 18 i.e. *Eriobotrya japonica* which neither modulate water content nor lipid content but still show signs of acclimation as evident from decrease in REL(%). This indicates that this species also has certain different mechanism of fighting low temperature stress which needs further investigations. From the above discussed results it has been confirmed that many of the subtropical plant species acclimate to frost induced freezing stress by way of adjustments in their water, lipid or both water and lipid content. But, there are some species which neither modulate water content nor lipid content but still survive severe frost conditions due to some other intrinsic mechanism which need further investigations. The degree of acclimation acquired by different species is different that is why that certain species though adjust their water and lipid content but still suffer certain level of frost damage. The species which do not modulate their water or lipid content mostly suffer frost damage except few species like *Eriobotrya japonica* which has shown decrease in REL% without modulation of water or lipid content, further investigation on low temperature behavior of this species will also be carried out in near future.

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