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Use of thermal hysteresis inducing chemicals for frost protection in subtropical fruit plants

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ABSTRACT : Frost induced freeze damage is the severest environmental stress faced by the subtropical plant species worldwide. Controlled environmental studies were conducted at Institute of Biotechnology and Environmental Science Neri for using thermal hysteresis inducing chemicals as a tool for managing this stress in highly sensitive, medium sensitive and least sensitive subtropical fruit species. Foliar mist of 5 per cent 1, 2-Propanediol was found most effective treatment in reducing the relative electrolyte leakage in the experimental plants. Ingression of this chemical into plant system through hydrogel application in the effective root zone was found to give prolonged protection against frost.

KEY WORDS : Mango, Papaya, Freeze initiation, Low temperature stress, Freeze restriction, Ice nucleation, Hydrogel

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Recent changes in the timing of environmental events attributable to climate change have had important repercussions for agriculture worldwide. As per the earlier trends of weather parameters the plants start ceasing their physiological activity as the winters advanced. Contrarily, as per the recent trends the rise in temperature during winters initiates physiological activity and the acclimation of the plant system to low temperature gets interrupted especially in case of subtropical fruit plant species and it exerts very devastating effects by way of increasing the vulnerability of the plant species to the frost and low temperature stress in the North-West subtropical region. Frost induced freezing is the severest plant stress as it causes ice formation, dehydration and cell deformation. It is not the cold temperature but ice formation that actually injures the plants. Much research published during the previous two decades concentrated on the intrinsic and extrinsic movement of water and ice growth during a frost event. As far as frost protection methods are concerned, the efficacy of the traditional methods such as fogging, smudging, windmill, overhead sprinkling etc. has always been under question mark. During seventies and eighties efficient technology was developed for frost protection through application of aqueous foams against radiative night cooling (Braud and Jerry, 1970; Siminovich *et al.*, 1972 and Krasovitski *et al.*, 1999). This technique restricts orchard heat transfer by spreading a foam layer over the orchard. But, due to its bulkiness and poor cost effectiveness this technique could not gain momentum even in the developed countries. It has now been realized that the strategies, which can delay or prevent initiation of ice formation, growth of ice crystal and spread of freeze within the plant system may prove better alternative for frost protection. Groenzin and Shulty (2008) illustrated through sum frequency generation spectroscopy that surface of ice crystal is highly reactive and the reactivity is influenced greatly by particular face (basal or prism) exposed. This supports the earlier views of Wilson (1994); Jorov *et al.* (2004)

and Madura *et al.* (2003) who opined that the thermohysteric compounds decrease the non- equilibrium freezing of water, by directly binding to the surface of an ice crystal (non-basal face), thereby disrupting the normal structure and growth pattern of the ice crystal. It not only inhibits the further ice growth but also prevent recrystalization of ice and thus, imparting thermal hysteresis *i.e.* imparting a change in the freezing point of cellular fluids. Present studies were, therefore, designed for testing the effect of thermal hysteresis inducing chemicals on prevention of frost induced freezing damage in the subtropical fruit species.

Horticulturally, mist or foliar application of the chemicals has been found to be the most convenient way of applying the chemicals to the target plants. But, while working on the above said thermohysteric experiments it was observed that this method of application was having certain limitations in respect of the use of the thermohysteric compounds. First limitation observed was that the mist/ foliar application gave effective results when applied just before the onset of frost event. Under natural conditions we can't guess the exact time of ice nucleation or freeze initiation and spread, hence, either the spray or mist applications is required to be continued throughout the night or we are required to have automated spray equipments which could either deliver thermohysteric compounds as intermittent mist or at the time of ice nucleation if we have proper prediction mechanism. Added to it, there is problem with the size of the trees to which application is to be made. Most of the commercial orchards happen to be more than 5 meters in height and therefore, require machinery of heavy capacity for making effective spray. Further, these spray applications give effective protection mainly for a single frost event. Keeping these problems in view, the studies were extended for working out alternative thermohysteric compound delivery mechanism into the plant system for achieving prolonged and more efficient frost protection.

RESEARCH METHODS

The studies were conducted under controlled environment simulated for radiation frost event at -2^{0}C air temperature at Institute of Biotechnology and Environmental Science during the years 2012 to 2015. Papaya, mango and loquat were the fruit species selected for the studies under highly frost sensitive, medium sensitive and least frost sensitive species as per categorization of these species done by Sharma and Badiyala (2008). Container grown two to three year old plants of mango and loquat and six month old plant of papaya were taken for the studies. In order to reduce the error of experimentation imparted by the intrinsic factors, the plants were exposed to the seasonal course of acclimation under natural conditions and therefore, experimentation was carried out during the month of December. There were taken eight treatments of thermal hysteresis inducing compounds as per Completely Randomized Design. These treatments were: T_1- Ethylene glycol @5 per cent (EG), T_2- 1,2 propanediol @5 per cent (PG), T_3- Sodium starch glycolate @ 1 per cent (SSG), T_4- Ethanol @5 per cent (EA), T_5- Methanol @5 per cent (MA), T_6- Warm water (50^{0}C), T_7- Cold water (10^{0}C), T_8 - Control – No application. The radiation frost conditions were simulated in the growth chamber at a temperature of -2^{0}C. The frost induced freeze initiation and spread was monitored with FLIR E-40 infrared – thermal video camera used for visualizing the exothermic events during the process of ice nucleation and spread. The mode of application of the treatments was misting of the canopy of the experimental plants with hand held sprayer as the first instance of freeze initiation was observed. The plants were continued further inside this radiative cooling environment for 2, 4 and 6 hours. The extent of frost induced freeze damage to the experimental plants was measured through the relative electrolyte leakage (REL) and drying- rehydration methods as described by Soliemani *et al.* (2003) and Sharma (2012 and 2015). The statistical analysis of the data were undertaken as per Completely Randomized Design as described by Gupta and Gupta (1995).

Further, for achieving prolonged and more efficient frost protection, experiment was conducted on testing of different methods for ingression of thermohysteric compounds into plant system. The most effective chemical shortlisted from the above experimentation (PG : 5% 1,2-Propanediol) was engrossed into plant system as per following treatments: D_1) –drenching of the root zone (500ml solution per plant), D_2) - placement PG saturated hydrogel beads (containing 500 ml of solution) in the effective root zone, D_3) - band application by wrapping PG soaked cotton around the plant stem where 2cm wide strip of bark have been removed upto ½ of the circumference just 5cm above the collar region and D_4) - Untreated control. The studies were conducted in a Completely Randomized Design on container grown mango plants of two to three years of age. All the experimental plants were treated once as per treatment details and were subjected to frosty conditions at -2^{0}C for 2, 4 and 6 hours in a single frost event. Each treatment was evaluated for 5 number of simulated frost events which were given at two days interval. The plants which got damaged during experimentation were replaced and were not subjected to experimentation again. The extent of freeze damage was accessed as per relative electrolyte leakage method as described above. The statistical analysis of this Completely Randomized Design was done as per procedure described by Gupta and Gupta (1995).

RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Experiment 1:

The data pertaining to effect of different chemical treatments on relative electrolyte leakage (%) observed in different fruit species exposed to different duration of freezing temperature exposure are presented in Table 1. The results are inferred as follows:

Papaya:

The effect of different chemical treatments on the relative electrolyte leakage was found to be non-significant in this case. However, the effective of duration of low temperature exposure was significant upto two hours of exposure level beyond which the effect on relative electrolyte leakage was found to be non-significant. This might have happened due to the fact that there happened almost complete damage to the plant foliage at four hours of exposure level to the freezing temperature. The interaction studies has however, revealed that treatment T_2 followed by T_1 and T_4 were very effective in reducing REL value upto 2 hours of freezing temperature exposure. Beyond this though these treatments do exerted their impact on reduction of REL but it was not statistically lesser than the REL value observed for control and other treatments. From these findings it can be concluded that in case of frost sensitive fruit species like papaya limited frost protection can only be achieved with the application of thermohysteric compounds. Application of T_2 *i.e.* 5 per cent 1, 2 propanediol has been found to be most effective followed by T_1 (Ethylene Glycol @5%), T_4 and T_5 (Ethanol @5% and Methanol @5%).

Mango:

The lowest REL value was observed at exposure level of 2 hours and it significantly increased with the increase in duration of exposure to freezing temperature. The effect of different thermohysteric compounds on REL (%) was observed to be significant in comparison to control and water treatments. The lowest value of REL was observed with T_2 followed by T_5, T_1, T_4 and T_3. The differences observed amongst these treatments were found to be statistically at par. The interaction of duration of exposure and the chemical treatments revealed that upto two hours of exposure to freezing temperature all the treatments exerted similar effect on REL. It implies that with foliar application of water same level of protection can be achieved as any of the thermohysteric compound can impart. At four hours of exposure the REL value observed with T_2 followed by T_1, T_5 and T_4 was far lower than that observed with other treatments and control. It means that at this level of freezing temperature exposure significant protection against frost can be achieved with foliar application of thermohysteric compounds (T_1 to T_5). But, best results were observed with T_2 (5% 1, 2 propanediol). Similar effect was observed at exposure level of six hours.

Loquat:

The loquat plants were very rarely affected by freezing temperature upto two hours of exposure. There was observed no electrolyte leakage in plants treated with different thermal hysteresis inducing treatments except the water treatments where some damage was observed. Considerably low value of REL was observed with different treatments in comparison to control and water treatments. Upto 4 hours of freezing temperature exposure there was not observed significant difference in the REL values. The REL values at 6 hours of exposure were significantly higher than the lower levels. Here also the lowest values were recorded with T_2 followed by T_3, T_1, T_4 and T_5. It means the foliar application of 5 per cent 1,2 propanediol was found effective in this case also.

Overall inferences drawn from the experiment indicates that it incase of papaya application of thermohysteric compounds was effective only when the frost incidence was of lower duration *i.e.* for 2 hours. In case of mango it was found that the frost incidence of low duration can be managed with water application. But, for longer duration frost this treatment was not found very effective and under such circumstance application of thermohysteric chemical was found very effective. Amongst these chemical treatments foliar application of 5 per cent 1,2 propanediol was found to be most effective followed by ethylene glycol @5 per cent, ethanol @5 per cent, and methanol @5 per cent treatments. In case of loquat, there has not been found any need of application of any treatment for protection against frosts of duration upto four hours. There occurs some damage during longer duration frosts and this can be managed as per the treatments described best for mango. Use of thermal hysteresis has also been studied by other workers in plants. Rubinsky and Devries (1992) patented antifreeze glycopeptide compositions to protect cells and tissues during freezing. They elaborated the compositions of substances, e.g. thermal hysteresis protein which are useful to improve survival, functionality and/or structural integrity in biological materials, e.g., micro-organisms, animal cells, tissues or organs exposed to freezing temperatures. Until recently, it was thought that all biological large-molecular-mass antifreezes were proteins. Now there are number of research findings which have elaborated the use of non- protein compounds for inducing thermal hysteresis which can provide cold resistance and freeze resistance properties when applied to a surface or a living system through a topical carrier. Walter *et al.* (2013) invented anti freeze glycolipid compound and composition which can be used for a

Table 1 : Effect of thermohysteric compounds and duration of freezing temperature exposure on relative electrolyte leakage (%) in different subtropical fruit species

Treatments (T)	Plant species (S)											
	Papaya				Mango				Loquat			
	Duration of exposure (E)				Duration of exposure (E)				Duration of exposure (E)			
	2hr	4hr	6hr	Mean	2hr	4hr	6hr	Mean	2hr	4hr	6hr	Mean
T_1	65.2	87.3	100	84.18	6.15	32.1	76.4	38.21	0.00	0.00	7.12	2.37
T_2	62.3	89.7	100	84.03	4.11	30.7	71.2	35.33	0.00	0.00	1.12	0.37
T_3	100	100	100	100.0	7.14	50.7	81.2	46.36	0.00	0.00	6.69	2.23
T_4	65.2	93.1	100	86.12	2.79	36.2	81.2	40.07	0.00	0.00	13.12	4.37
T_5	72.3	100	100	90.78	6.00	34.3	72.2	37.50	0.00	0.00	20.34	6.78
T_6	67.6	100	100	90.78	6.14	63.5	96.3	55.31	0.00	16.2	34.78	16.99
T_7	87.1	100	100	95.71	9.11	63.6	96.6	56.47	0.00	14.4	33.76	16.04
T_8	100	100	100	100.0	28.6	65.5	93.3	62.46	6.52	27.5	34.77	22.94
Mean	77.49	96.27	100	91.25	7.24	47.07	83.6	45.97	0.92	7.26	19.34	9.17
C.D. (P=0.05)	E = 14.21				E = 23.45				E = 11.25			
	T = NS				T = 13.43				T = 8.47			
	T*E = 17.12				T*E =14.72				T*E = 11.12			
	E*T*S =29.31											

Table 2 : Effect of method of application and number of frost events of different duration on REL (%) in mango

Treatments (D)	Frost events (FE)																			
	FE-I				FE-II				FE-III				FE-IV				FE-V			
	2hr	4hr	6hr	Mean	2hr	4hr	6hr	Mean	2hr	4hr	6hr	Mean	2hr	4hr	6hr	Mean	2hr	4hr	6hr	Mean
D_1	7.14	40.7	71.2	39.7	19.4	52.3	81.6	51.1	27.1	60.5	81.2	56.3	28.4	62.3	80.1	56.9	31.9	65.8	80.1	59.3
D_2	2.79	36.2	51.2	30.1	7.91	38.2	53.7	33.3	9.36	43.2	56.9	36.5	12.9	47.2	66.7	42.3	24.6	48.2	71.4	48.1
D_3	14.1	50.7	76.2	47.0	24.1	48.2	77.4	49.9	22.3	41.8	66.4	43.5	24.1	48.2	67.4	46.6	32.9	67.6	76.7	59.1
D_4	26.4	63.5	86.3	58.7	26.4	63.5	84.3	58.1	27.2	66.3	85.1	59.5	25.9	65.5	84.3	58.6	35.7	67.2	82.3	61.7
Mean	12.6	47.8	71.2	43.9	19.4	53.1	74.3	48.9	24.0	55.5	72.4	50.6	25.3	58.3	74.6	52.8	33.8	64.7	77.6	58.7
C.D. (P=0.05)	FE = 6.73																			
	D =7.60				D =8.21				D =8.60				D =7.72				D =11.60			
	E =9.81				E =7.81				E =15.81				E =12.81				E =15.81			
	FE*D =13.67				FE*D =11.27				FE*D =13.67				FE*D =11.67				FE*D =13.67			
	FE*E =13.54				FE*E =12.40				FE*E =13.54				FE*E =10.54				FE*E =13.54			
	D*E =6.41				D*E =7.74				D*E =8.11				D*E =4.23				D*E =12.41			
	FE*D*E =3.64																			

variety of industrial, agricultural or cryoprotection uses. The compound has been found very useful in protection plants from frost bite. Savignano and Hanafin in 1997 patented a process for preventing or retarding frost formation on substrate like leafy plants by surface application of a composition of water, a water soluble freezing point depressant like propylene glycol and water dispersible thickening agent. Significant achievement in protecting lawn grass with the use of thermal hysteresis inducing compounds has also been reported by Zollinger (2009) who claims protection for 4 to 6 weeks with single spray of such compounds on to plants surface. In the present studies 5 per cent 1, 2-propanediol has been found to significantly superior to other treatments. The properties of this compound as freezing point depressant have been well documented. The freezing point of water is depressed when mixed with propylene glycol owing to the effects of dissolution of a solute in a solvent (freezing-point depression); in general, glycols are non-corrosive, have very low volatility and very low toxicity, however, the closely related ethylene glycol (a key ingredient in antifreeze) is extremely toxic to humans and fatally toxic to many animals. Propylene glycols are most commonly used in applications in which low acute oral toxicity is required, or for freeze protection where incidental contact with drinking water is possible. Propylene glycol based fluids are used extensively in food processing application. The 5 per cent aqueous application through hydrogel into the container grown mango plants have shown significant reduction in frost induced freeze damage. However, there are certain findings which report the poor response of the anti freeze chemicals in plants especially in annuals. In a study Perry *et al.* (1992) reported that a commercially available formulation (50% propylene block copolymer of polyoxyethylene, 50% propylene glycol; trade name FrostFree) and an antitranspirant (96% di-1-p-menthene, *i.e.*, pinolene, a terpenic polymer, 4 % inert; trade name Vapor Gard) when for their ability to protect ‘Pik Red’ tomato (*Lycopersicon esculentum* Mill.) and ‘Keystone Resistant Giant #3’ pepper (*Capsicum annuum* L.) plants during frost and freeze occurrences in the field were not able to give protection under field conditions when minimum air temperature reached -3.5^{0}C.

Experiment 2:

The data pertaining to the effect of different treatments on the REL (%) observed during different freezing durations of frost events are presented in Table 2. The REL value increased as the experiment proceeded from frost event I to frost event V but the increase observed was non-significant amongst the frost event. It implies that the REL value of the previous frost event did not influenced the REL value of the proceeding event. During a frost event as the duration of low temperature exposure increased, the REL value also increased significantly. It was observed uniform amongst all the frost events studied. It reflected that the REL value was more a function of duration of low temperature exposure rather than the number of frost event observed by the plants. During the first frost event it was observed that lowest value of REL was observed with the treatment D_2 followed by D_1. The interaction studies revealed that the treatment D_2 maintained the lowest value of REL upto 6th hour of low temperature exposure whereas the effect of D_1 faded after 4th hour of exposure to freezing temperature. D_2, therefore, happened to be the best treatment during first frost event. Likewise during second frost event the lowest value of REL was recorded with D_2 but the second lowest were recorded with D_3 instead of D_1 though these two treatments were statistically at par with each other. It might have happened due to the fact that the quantity of the applied chemical through drenching (D_1) might have exhausted with the passage of time which resulted in higher value of REL under D_1. Very similar effect of treatments was observed during the third frost event also but the effect of different treatments was superior to the control. During the fourth event also the lowest value of REL was recorded for D_2 followed by D_3 and D_1 but, none of the treatment except D_2 differed significantly from control, means the damage level observed under D_1 and D_3 was not significantly lower than the damage observed under control treatment (D_4). During the 5th frost event the lowest value of REL was observed under D_2 followed by D_3 and D_1 but it was not significantly lower than the value of REL observed under the control. It implies that treatment D_2 also could sustain its effect upto 4th frost event but not upto the 5th event. Therefore, from these it has been concluded that D_2 *i.e.* placement PG saturated hydrogel beads in the effective root zone was found to be the best treatment for achieving considerable frost protection in mango plants and it was effective for much longer time than the other methods of thermohysteric compound ingression into the plant system. The term hydrogels was originally introduced by Wichterle and Lim in 1960s and its biological application was put forward. Since then, hydrogel technology has evolved at a huge scale in pharmaceutical industry and to small scale in agriculture. The term hydrogel is self explanatory for their existence, since the evolution of life on earth. The structure of plants, the components of extracellular matrix, the bio-films of micro-organisms are everywhere, all the swollen moieties in nature are the proofs of their occurrence. The first paper sighted was by DuPont scientist in 1936 for medical applications, which introduced the spark that was enlightened in 1960 by Wichterle and Lim (1960) who worked on poly (2-hydroxyethylmethacrylate) poly (HEMA). Hydrogels are defined as a three dimensional biopolymeric networks, which have the tendency to absorb large quantity of water and they themselves are not soluble in water. This led to the keen interest in hydrogels as a class of biomaterials and their application as drug delivery systems. In plants and agriculture these hydrogels are used as a slow-release source of water and dissolved salts into the soil and thus have been found to increase growth and yield in number of agricultural crops (Vundavallia *et al.*, 2015). The emphasis of the present study was to highlight the use of these synthetic polymers for delivery of thermohysteric compounds into the rhizosphere for prolonged duration of time so as to harness the effect of the hydrogel for sustained protection against frost.

Conclusion:

Thermal hysteresis inducing compounds have significantly reduced the electrolyte leakage in all the frost sensitive and frost tolerant plants. Foliar application of 1, 2 propanediol (Propylene glycol) @5 per cent has been found to be best treatment for frost damage reduction followed by ethylene glycol, ethanol and methanol application at the same rate. Incorporation of hydrogels saturated with 5 per cent 1, 2 propanediol has been found to give prolonged protection in comparison to the other methods of ingression of the compound into plant system.

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