novel and reproducible protocol for germination and development of orchids. This protocol is a viable technique for large-scale multiplication of orchids.

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Impact of chilling injury on common fruit plants in the Doon Valley

The response of plants to the severities of the environment have occupied the attention of man long before the beginnings of the science of biology[1]. Biological stress may be defined as any environmental factor capable of inducing a potentially injurious strain in living organisms, which may be either a physical or chemical factor, however, any of these factors occur in severe form which lead to permanent damage to plant or organism. Plants are poikilotherms – they tend to modify their body temperature according to prevailing temperatures at their site. Tropical and subtropical plants exhibit a distinct physiological damage when they are exposed at low temperatures below 10°C (ref. 2). This is referred to as 'chilling injury'. It is observed at temperatures above 0°C and is quite distinct from freezing injury. This is mainly associated with physical disorders caused by freezing of water. Although chilling injury can be induced in complete darkness, it is more marked under weak light[3]. Chilling injury in light is regarded as a kind of photoinhibition[4]. Chilling injury can occur under certain distinct characteristics of lower threshold temperature below which it is induced in the plant[5,6]. It causes damage to the chloroplast and affect photosynthetic activities which are irreversible in nature, and leads to reduction in the rate of photosynthesis. Thereafter, visible symptoms develop in the plants after several days of hidden chilling injury.

Chilling injury may lead to indirect damage to plants. These may include starvation, in which the respiration rate may exceed the rate of photosynthesis, which gets reduced due to damage to thylakoids[7], or by inhibition of translocation[8]. Other reasons include accumulation of cell toxins[2] and biochemical lesions[9]. Recent work indicates that among other processes, paired redox and arcadian regulation may be influencing photosynthetic processes during low temperatures[10]. A study on *Eucalyptus pauciflora* Sieb. ex Spring seedlings reported that elevated atmospheric CO_2 promotes frost damage to evergreen tree seedlings[11].

Low temperatures and chilling injuries are fairly common in the subtropical regions where plants have adapted themselves to withstand low temperatures, but more recently shifts in weather patterns have been noticed. The occurrence of frost in areas adjoining the Himalayas at lower elevations and also in locations far away from the mountains has become frequent, leading to large scale loss of crops, fruits and vegetables.

Pool frost (convection frost) is defined as 'the accumulation to a considerable depth of heavy cold air flowing down into natural depressions from adjoining mountain areas'. This has more deleterious effect on vegetation since the freezing effect extends to a considerable height. This occurs when 'heavy', cold air from the mountains moves down into valleys and settles down for longer periods (Figure 1 *a*). This cold air is removed only by heating effect of the sun. On the other hand, advective frost is defined as 'a frost produced by cold air brought from elsewhere' (Figure 1 *b*). While the former type is of frequent occurrence in hills and valleys, the later occurs extensively in Northern India, especially in the plains of Punjab, Haryana, Delhi extending up to Rajasthan.

Here we report the impact of chilling injury on fruit plants in the Doon Valley, as a result of low temperatures induced by pool frost that occurred in the winters of 2002–2008.

Assessment of the extent of chilling injury was carried out by field surveys, during spring in 2003, 2007 and 2008. The Dehradun Valley (77°40′–78°15′E and 30°00′–300°35′N) lies in the subtropical climatic region between the West Himalayan mountain ranges in the north and the Shiwalik range running parallel to it in the south, covering an area of 1920 sq. km, with a mean elevation of 985 m asl. Climatically the valley experiences annual mean maximum (29.11°C) and mean January temperature (13.78°C), with an average rainfall of 1625.28 mm. The valley drains out into the Yamuna in the west and into the Ganga in the east. Due to its close proximity to the Himalayas, the valley experiences severe winters during December and January. Summers (May–June) are warm and dry, followed by a fairly distributed rainfall (mid-July to early September) and winter sets in by mid-October. Due to the favourable climatic conditions, well drained deep alluvial soils, the Doon Valley is well-known for its Mango, Litchi, Citrus, Peach, Guava, Papaya and Aonla orchards and fruit tree-based agroforestry practices. A number of crops are grown in these orchards as understorey crops, both under irrigated and rainfed conditions, ranging from wheat, rice toria, pulses, vegetables and marigold. Among the various fruit types, mango-based agroforestry practices are the most popular (57.8%), followed by litchi (15.6%), guava (11.2%), kinnow (7.8%), peach (4.4%) and citrus (1.1%).

Field surveys were carried out in the spring months to assess the effect of 'extreme' winter conditions. Surveys were carried out in four blocks of the valley. A total of 37 orchards of different fruit types were surveyed in 21 villages located in Sahaspur, Vikasnagar, Raipur and Doiwala blocks of Dehradun district. While the first three are situated in close proximity (< 6 km) as the crow flies to the Himalayas, Doiwala is on the fringe of the Doon Valley and is probably not affected by the occurrence of pool frost in the valley per se.

Data collected on plant mortality in various fruit species existing in four blocks were statistically analysed using a randomized block design (RBD) with four factors (age, intercrops, windbreak and elevation) with four replications (blocks were treated as replications).

The Doon Valley is well known for its salubrious climate all through the year; however, December and January are the coldest months. Thirty years (1972–2001) average maximum and minimum temperature during December and January were 20.2°C, 4.2°C and 22.6°C and 4.5°C respectively. However, during December 2002 and January 2003, the minimum temperatures were unusually low. During the last week of December 2002, the minimum temperature varied between 1.2°C and 1.5°C against a 30 years average of 3.7–3.9°C (Figure 2 *a*). Similarly during 10–14 January 2003, a temperature of 1.0°C was recorded against a 30 years average of 4.3°C. These cold-wave conditions continued till 26 January 2003 after which there was a gradual increase in temperature. During this period, the duration of bright sunshine hours were 0.7–2.1 h less than the 30 years average (6.0 h). During December 2007, January 2008 and February 2008, temperatures continued to remain low (Figure 2 *b*) which were lower than the 30 years average, although sunshine hours were more or less the same during 2007–2008. Overall, a general lowering of winter temperatures and that too for a longer period was observed in 2008 in comparison to 2003.

Chilling injuries were manifested in several forms in mango, litchi, guava, aonla and papaya.

Mango: Data revealed that maximum cumulative mortality (78.4%) occurred at lower elevation (< 500 m asl) followed by 61.2% at mid-elevation (500–600 m asl) and minimum mortality 17% was recorded at higher elevation (> 600 m asl). This reveals that plants growing at higher elevations suffer

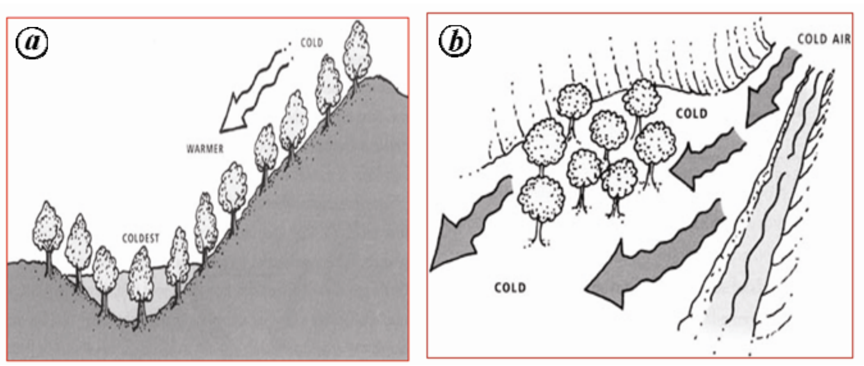

Figure 1. ***a***, Occurrence of pool frost in areas adjoining high mountain ranges. ***b***, Occurrence of advective frost in areas far away from mountains.

minimum damage on account of higher temperatures in comparison to those occurring in the valley, which may be attributed to the occurrence of pool frost, where cold air remains confined to lower elevation. Result of mortality in different age group of orchards revealed that the highest percentage of death (94%) was reported in the young age group (< 2-year-old) with lowest percentage of death (8.5%) occurring in older plantation (> 6-year-old). While comparing the sole orchard (mango only), mixed orchard and mango with intercrops, the data revealed maximum plant mortality (87%) has taken place in sole orchard, whereas minimum mortality (28%) was recorded in mango plants grown along with intercropped crops. Mango plants grown in combination with woody perennials and annual intercrops were less affected to some extent against low temperature in comparison to pure mango orchards. Barriers of any kind – biological (wind breaks, shelter belts) and engineering (cement structures, tall buildings, high compound walls) have a marked influence on microclimate of a place. The results of the study revealed that minimum mortality (19.44%) was observed in orchards surrounded by wind breaks, whereas in the absence of these barrier led to 90.5% mortality in mango.

The susceptibility of mango plants to abnormal winters were studied for three winter seasons, with respect to elevation differences, age group, orchard composition and physical barriers. Mango was the second most susceptible fruit plant, in spite of its wide adaptability to a range of elevations extending up to 1400 m asl. Mango needs an optimum temperature range of 24–26.7°C and minimum threshold temperature is 10–12°C below this plant shows chilling injury[10]. Stomatal density on mango leaves is high on the upper layer, making them susceptible to low temperatures. Singh and Singh[12] reported that a minimum temperature of – 0.6–0°C for 1 h and 15 min for two consecutive days resulted in significant damage to mango trees.

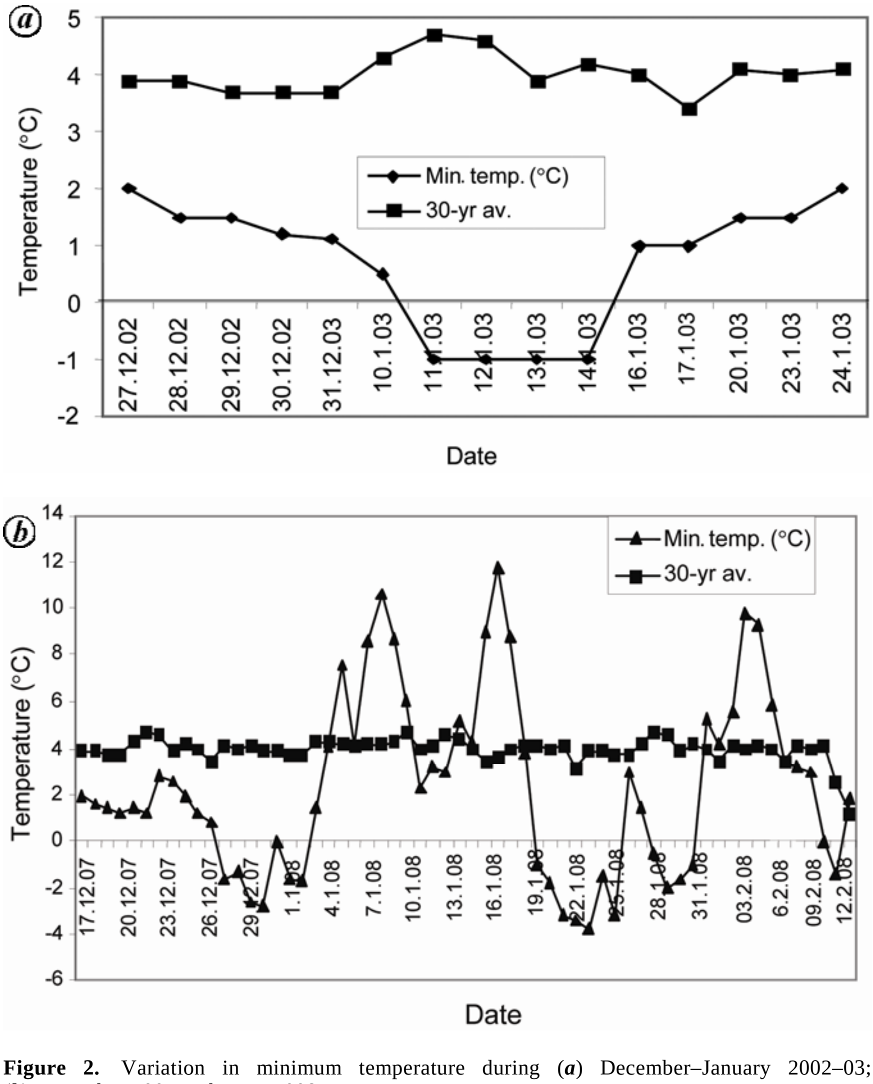

Figure 2. Variation in minimum temperature during (***a***) December–January 2002–03; (***b***) December 2007–February 2008.

Litchi: Effect of chilling injury in this fruit indicated that mortality rates were nearly 10% at elevation of 500 a msl and reduced with increasing elevation (> 600 m; Table 1). Young plants (<2 years) showed maximum mean mortality (12.6%) and minimum mortality (3.15%) in older age group (> 6 years) plants. Mortality in litchi plants under different category of orchards revealed that pure litchi orchards were the worst affected (21%) and litchi plants with intercrop were the least damaged (6.15%). Data recorded on plant mortality with different type of barriers indicated that maximum mortality (30.4%) was observed in litchi plants without any barrier, whereas least mortality (3%) was noticed in litchi plants surrounded with wind breaks. The existence of wind breaks led to significant reduction in mortality in comparison to other fruits like mango, guava and papaya.

Unusually low temperatures accompanies with frost led to the death of young plants, although older trees (> 6 years) withstood the damage, with low mortality rates. Litchi plants were least affected amongst all fruits studied because they require a lower threshold temperature (< 6°C) for initiation of flowering and fruiting. The plants also contains oil in the plant body which save the plant from low temperature injury, but temperatures below 0°C have damaged the plants as well as reduced fruit yield up to a certain extent.

Guava: Trends in analysis of mortality rates among the four factors in guava showed that barrier and orchard category had the major influence in death of plants followed by age group and elevation. Maximum mortality rates were recorded as 43.34%, 41.16%, 36.8% and 32.35% in open orchard (no barrier), pure orchard, < 2-year-old orchard and 500 a msl elevation respectively. While minimum damage and mortality rates of 4.53%, 6.1%, 9.8% and 12% were observed in more than 6-year-old plantation, orchard with intercrops, plantation above 600 m elevation and orchard surrounded by wind breaks respectively.

Table 1. Mortality (%) of different fruit plants affected by frost in Doon Valley (2002–08)

Parameter	Category	Mango	Litchi	Guava	Aonla	Papaya
Elevation (m asl)	< 500	78.39	9.60	32.35	80.16	79.93
	500–600	61.18	6.83	19.90	67.93	67.45
	> 600	17.02	4.38	9.78	36.18	31.73
CD (*P* = 0.05)		9.86	4.18	3.98	9.23	8.46
Age group (years)	< 2	94.09	12.59	36.78	95.85	95.69
	2–6	31.45	6.77	10.98	53.44	50.54
	> 6	8.47	3.15	4.53	33.82	28.47
CD (*P* = 0.05)		3.82	5.04	4.95	6.33	5.61
Orchard category	Pure orchard	86.96	21.06	41.16	88.06	88.29
	Mixed orchard	67.91	9.10	17.68	73.77	70.58
	With intercrops	28.02	6.15	6.11	43.15	39.51
CD (*P* = 0.05)		8.77	8.05	4.11	8.45	9.12
Barriers	Wind breaks	19.44	2.97	11.98	28.30	30.81
	Physical barriers	32.61	8.92	16.41	41.92	42.74
	Open	90.50	30.43	43.34	91.43	90.81
CD (*P* = 0.05)		8.80	5.92	4.82	8.67	6.55

The presence of scale bark and cork layer in the stems of guava plants provides some protection against marked variations in temperature, although leaves in most plants were killed immediately, this being attributed to large number of stomata on the upper leaf surface. Mature plants of guava are frost hardy and can tolerate low temperature (< 5°C). The essential oil in the plant body helps the plants to withstand the low temperatures.

Aonla: Mortality percentage of aonla plants ranks high in general for all factors under study. In the lower elevation, death of plants was conspicuous (80.16%) followed by mid-elevation as compared to higher elevation (36.2%). Success of any plantation/orchard depends upon the number of young plants/ seedlings growing in to a tree. In this case mortality rate were 95.85%, 53.44% and 33.8% in less than 2-year-old plantation, 2–6-year-old and more than 6-year-old respectively. It revealed that overall mortality percentage in this factor was higher as compared to the other species studied. This is the one of the reason which limits the establishment of aonla plantation under Doon Valley conditions. Orchard categories had limited influence in reducing the mortality of aonla plants. The results revealed that maximum (88%) mortality was observed in pure orchard followed by mixed orchard, whereas minimum (43.15%) was recorded in aonla with intercropping. Aonla plantation protected by wind breaks recorded the least mortality (28.3%) compared to the unprotected plantation which recorded 91.4% mortality.

The upper and lower threshold temperatures for proper growth and development of aonla were 50°C and 12°C respectively and beyond which, aonla plant ceases to grow[13]. Aonla is a subtropical fruit suitable for the arid zone due to its plant ideotype and is sensitive to low temperatures[14]. Aonla fruit is suited for dry and moisture stressed condition by changing its morphological features like thicker cuticles, palisade tissues and lenticels, which make aonla suitable for cultivation under arid climate. These morphological features aggravate frost susceptibility to aonla plants under subtropical frosty conditions. The reasons for frost susceptibility are lower concentration of solutes, inelastic protoplasm, water does not initiate metabolic activities after melting of ice crystals and vacuoles do not split into number of small vacuoles to reduce turgor pressure. Higher number of lenticels are present in aonla plant. As the number and size of lenticels increases, the epidermis gets ruptured and gas exchange takes place between the plant and the environment. Damage is visible in the form of burning and scorching of leaves, death of twigs and branches and splitting of main trunk.

Papaya: All cultivars are susceptible to low temperature, therefore, its cultivation is restricted to limited areas in Uttarakhand. Field survey data revealed that maximum mortality (95.7%) occurred in orchards which were less than two-year-old followed by pure orchard grown in open areas. On comparing the impact of elevation level and orchard category, the latter has more mortality (88.3%) in plants than elevation level which caused 80% mortality at < 500 m asl. While minimum death percentage were 28.5, 31, 31.7 and 39.5 recorded in > 6-year-old orchard, orchard with wind break, orchards at elevation > 600 m asl and orchard with intercrops respectively.

Temperature is one of the most important factors which determine the success of papaya cultivation. Papaya is highly susceptible to extremes of temperatures and waterlogging. In addition to the above factors, it is affected severely by frost. Papaya plantations are severely damaged at 4.0°C temperature[13]. Minimum threshold temperature of papaya is 12°C below which papaya gets severely affected by low temperatures during the winter season. The exact mechanism of frost damage is poorly known. However, it usually damages the plant at the ground level in cold places. In such cases the bark of the young plant is damaged and cracks open following which the inner tissues carrying sap gets ruptured through freezing[15]. Cell sap of papaya is less viscous because of less solutes and more water, which makes it susceptible to low temperatures and also formation of intracellular ice-crystals. Nature of frost damage of different organs of papaya plant varies, but physiologically overall effect may be the burning of the whole plant. Stem injury is characterized by the oozing of latex from the stem surface about 24–45 cm above the ground level for a week, which leads to the rotting of stem and ultimately death of plants. Symptoms of leaf injury in the form of scorching is visible 10–12 h after the occurrence of frost, which becomes apparent after 20–24 h. Similarly, symptoms of frost injury on growing shoots, flowers and fruits can be seen 6–12 h after frost. Usually, mature fruits are less affected by frost than immature fruits[16,17].

Farmers can protect their standing fruit crops under frosty weather by creation of hot air or smoke screens, covering small fruit plants with straw, dry grass, etc. applying irrigation to maintain soil moisture in the soil profile and foliar spraying of water on the plants twice early in the morning. Wind breaks or shelter belts can also be raised around the existing plantation. This will moderate the abnormal climatic condition to a conducive condition by reducing cold wave damage, reduce wind speed and maintain optimum temperature in and around the orchards. It is also suggested that new orchard growers select low temperature tolerant fruit species and varieties like peach, sweet orange, mandarin, lemon, phalsa, loquat and kagzi lime, with a provision of wind breaks or shelter belts. The Doon Valley is suitable for the cultivation of fruits which are not affected by low temperatures. Citrus species, such as lemon and lime were damaged to an extent of <5% and the overall health of the plants remained unaffected. Loquat is an evergreen, subtropical and underutilized fruit plant, which is being grown on a limited scale by growers. These plants showed no damage and appeared to thrive better under low temperature. Phalsa also a minor, subtropical and winter deciduous fruit plant, remains under dormancy during December–February (chilling period) and did not show any symptoms of damage either on leaves and shoots. The resistance of these hardy plants depends upon species, leaf structure and concentration of solutes, as these reutilize water formed after melting of crystals for initiating metabolic activities in the cells, maintain higher concentration of solutes, cell protoplasm remains more elastic and vacuole splits into a number of small vacuoles to reduce turgor pressure of the cell.

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From data repositories to potential biomarkers: application to prostate cancer

The digital nature of RNA-seq technologies offer sensitivities in measurements that were not achievable by hybridization-based technologies; thus fast replacing two decades of genomics technologies, including gene expression microarrays, single nucleotide polymorphism (SNP) arrays, exon arrays and splice variant microarrays. Unlike microarray-based measurements, where one has the limited view of RNA composition through known genes, known splice events, known SNPs, or known exons represented on the microarrays, RNA-seq cracks open the cells exposing diverse RNA phenotypes in its entirety. Thus, RNA-seq technology is becoming a method of choice to measure context-specific translocation[1], gene expression[2], alternative splicing[3], single nucleotide variations (SNVs)[4], non-coding RNAs[5], transcript-induced chimera[6], and microRNAs[7] from a single experiment. This, together with the steep drop in sequencing costs, has made data analysis a bottleneck in biology. The NCBI repository is home to a large number of data