Manual
on
Phytotron for Speed Breeding and Precision Agriculture

Centre of Advanced Agricultural Science & Technology (CAAST)
National Agricultural Higher Education Project (NAHEP)
(ICAR- World Bank Project)
Protected Agriculture and Natural Farming (PANF)
and
Department of Genetics & Plant Breeding
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176062, H.P.

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PREFACE

The Himalayan region represents a unique agricultural ecosystem which is known for its richness in bio-diversity of plants, animals & microorganisms and natural resources such as water, forest, fertile soil, agreeable weather conditions, and breath-taking landscapes due to their various ecological niche and altitudinal variations. About 85 per cent of the Himalayan population relies directly or indirectly on traditional integrated hill agriculture, animal husbandry, agro-forestry and forestry for their livelihoods. It is now well understood and recognized that the development of superior high yielding, fertilizer responsive and climate resilient varieties with better nutritional properties is possible with the plant breeding and joint ventures of all other disciplines. Since, developing varieties with conventional breeding methods takes a lot of time (usually 10-15 years) to release a variety. This urges the scientists to develop a methodology to hasten the breeding procedures by reducing the time required to develop new cultivars which leads to the introduction of a new technique called Speed Breeding. In this context, Department of Genetics and Plant Breeding has been established novel rapid generation advanced facility "Phytotron facility" under the National Agricultural Higher Education Project– Centre of Advanced Agricultural Science & Technology (NAHEP-CAAST) subproject on "Protected Agriculture and Natural Farming (PANF)" funded by GoI and World Bank. The Phytotron facility allows researchers to access the majority of climatic situations, allows for a wide range of research activities, off-season hybridization, precise and effective data reproduction, make various environmental conditions in very small space and rapid generation advancement. The manual provides state-of-the-art information on phytotron facility to speed up plant breeding.

I earnestly feel that this manual will be highly useful for students, research scholars and scientists working in the area of plant breeding at universities, research institutes and R&Ds for conducting research and various funding agencies for planning future strategies.

I am highly grateful to all learned contributors each of who has attempted to update scientific information, expertise and has kindly spared valuable time and knowledge.

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Palampur
August, 2022

(H.K. Chaudhary)
Vice-Chancellor

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INTRODUCTION

Global food security has become a major worry as the human population grows and the environment changes, with the current rate of improvement of numerous vital crops insufficient to fulfill future demand. The following breeding techniques, in the order listed, can be are generally practiced in any crop improvement programme: (a) selection of desirable parents with complementary traits to combine; (b) crosses involving the selected parents and the development of progenies; (c) selection and genetic advancement of the best progenies based on target traits; (d) screening of the best progenies in multiple target production environments to identify the best performing and stable candidate cultivars; and (e) cultivar registration, seed multiplication, and distribution to growers. After crossing selected parent lines, 4–6 generations of inbreeding is usually necessary to establish genetically stable lines for agronomic trait and yield evaluation. This is especially time-consuming for field-grown crops, which typically only have 1–2 generations every year (Watson et al. 2018). The time spent in these stages considerably slows the development and marketing of cultivars. Speed breeding procedures can beutilized to create homozygous lines quickly by away of raising number of generations per year. Photoperiod, light intensity, temperature, soil moisture, soil nutrition, and high-density planting are all used in this strategy. Early flowering and seed set have been induced using these strategies, shortening the time it takes to develop each breeding generation. Three to nine breeding generations can be produced per year using this procedure. This is appropriate for fast breeding and population evaluation across target production conditions using single seed descent (SSD), single pod descent (SPD), and single plant selection (SPS) approaches (El-Hashash and El-Absy, 2019). Both conventional and genetically modified agricultural cultivars could benefit from speed breeding procedures. However, access to proper facilities, people educated in the protocol, implementing fundamental modifications to breeding programme design and management, and the necessity for long-term finance are the most typical roadblocks to expedite breeding's adoption. To adjust environmental parameters such as soil moisture, temperature and photoperiod, speed breeding platforms require complex infrastructure (Gosh et al. 2018). In many underdeveloped nations, institutional support for public plant breeding programmes is weak. This restricts the use of cutting-edge breeding techniques like speed breeding and biotechnology tools (Byerlee and Fischer, 2002).

A useful tool for such a type of study is phytotron. A phytotron is a collection of controlled-environment cabinets, rooms and glasshouses set up in such a way that many different combinations of environmental parameters can be investigated at the same time (Downs, 1980). The Phytotron facility allows researchers to access the majority of climatic variables while removing the variability associated with open environmental situations. The facility allows for a wide range of research activities, including germplasm maintenance, plant evaluation for various phenotypic and genotypic studies under various environmental regimes for the crop, off-season hybridization and multiplication and a one-of-a-kind venture to strengthen and accelerate the ongoing pre-breeding programme(Downs, 1980).Phytotron facilities assist to eliminate bias by generating reproducible data over the course of a year, allowing researchers to assess the feasibility of numerous innovative methodologies. Experiments can be set up in this way such that adjacent growing rooms have the same lighting, temperature control, humidity, day length, watering, mineral feeding regimes and other growing variables as needed (Downs, 1980). Plants grow in distinct sections and can be moved between the growing chambers, allowing for a greater number of combinations of different conditions to be tested. There have been several outstanding phytotrons built (Downs, 1980). Some, such as those found in Australia, France, New Zealand, and the United States, are multipurpose phytotron. Others are created for specific plant species, such as the rice research centre in the Philippines, or for specific research purposes, such as cold hardiness, as in the USSR and Hungary. The importance of phytotron in the overall strategy of biological research is demonstrated by the fact that at least 19 countries have one or more phytotron varying in size from 100 to over 600 m^2. The most recent of these facilities are in Canada, India and the Soviet Union.

The economy of the state Himachal Pradesh is highly dependent on three sources: hydroelectric power, tourism and agriculture. A wide range of agro-climatic conditions of Himachal Pradesh with temperature range of 0°-10°C (winter) and 15°-30°C (summer) provides ample scope to cultivate various important crops in Himachal Pradesh. There is great variation in the climatic conditions of Himachal due to extreme variation in elevation. The main cereals cultivated in Himachal Pradesh are wheat, maize, rice and barley. The agro-climatic conditions of Himachal Pradesh are also suitable for the cultivation of potato, ginger, soybean, oilseeds, pulses, fodders and off-season vegetables (Cash crops). The State Government also encourages the production of off-season vegetables, potato, ginger, pulses and oilseeds besides increasing production of cereal crops. In this way, by adopting a diversification approach, hill agriculture is going to be more and more remunerative day by day. Though, Himachal Pradesh provides favorable environment for raising almost all types of agricultural crops due to widely varied agro-climatic regions but the production and low productivity of different crops in the state is a major concern and a focused research is needed for the betterment of the agriculture sector. Upscaling traditional farming for native mountain crops and integrated research is a new necessity for agricultural research and development in Himachal Pradesh. Therefore, a potential research challenge is to work on new varieties and cultivation practices that fit in well with the changing climate scenario. The researchers currently face a major constraint with regard to studying of the plants in varied agro-climatic zones of the state for that they have to conduct multilocational experiments and the data generated from the experiments have lot of errors which affect the ultimate objective of the studies. The process is lengthy and not realistic. The solution for this is Plant Phytotron facility. Plant Phytotron is a facility where the climatic conditions from desert to the rainfed to the irrigated lands can be replicated.

FACILITIES

In modern agricultural research, a phytotron is an essential component of genetic, physiological and biotechnological crop development and protection applications. Agricultural scientists in India have been proposing the establishment of a controlled environment facility since 1966, and each decade since then has brought up the disadvantages in agricultural research progress if a Phytotron is not made available to India's plant scientists. The DST's Programme Advisory Committee on Plant Physiology and Biochemistry recommended again in 1983 that India establish a National Facility for Controlled Environments. Following that, the planning commission's steering committee on research, technology and the environment suggested that such a facility be established at the institution. The scientists of CSKHPKV, Palampur prepared a scientific proposal and officially submitted it to the ICAR, New Delhi, in 2020-21 under the National Agricultural Higher Education Project–Centre of Advanced Agricultural Science & Technology (NAHEP-CAAST) subproject on “Protected Agriculture and Natural Farming (PANF)” funded (about 86 lakh rupees) by Government of India and World Bank. Out of which one of the focus relies mainly on the establishment of phytotron. The project proposal was suitably modified in the light of comments from the committee and it was finally approved for implementation in August 2020-21.

The single-story Phytotron contains areas for general studies and for research in genetics and plant breeding, plant pathology, entomology and seed science. The facility contains eight controlled-environment chambers of various sizes (Figure 1), plus power supply chamber (Figures 2 and 3).

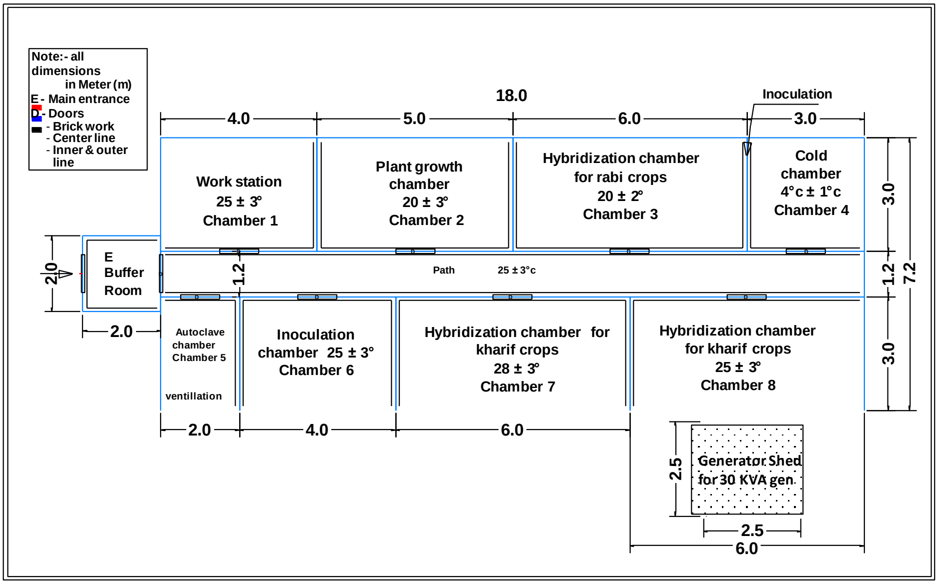

Figure: 1. Layout of CSKHPKV Phytotron facility

Figure: 2. Outlook of CSKHPKV Phytotron

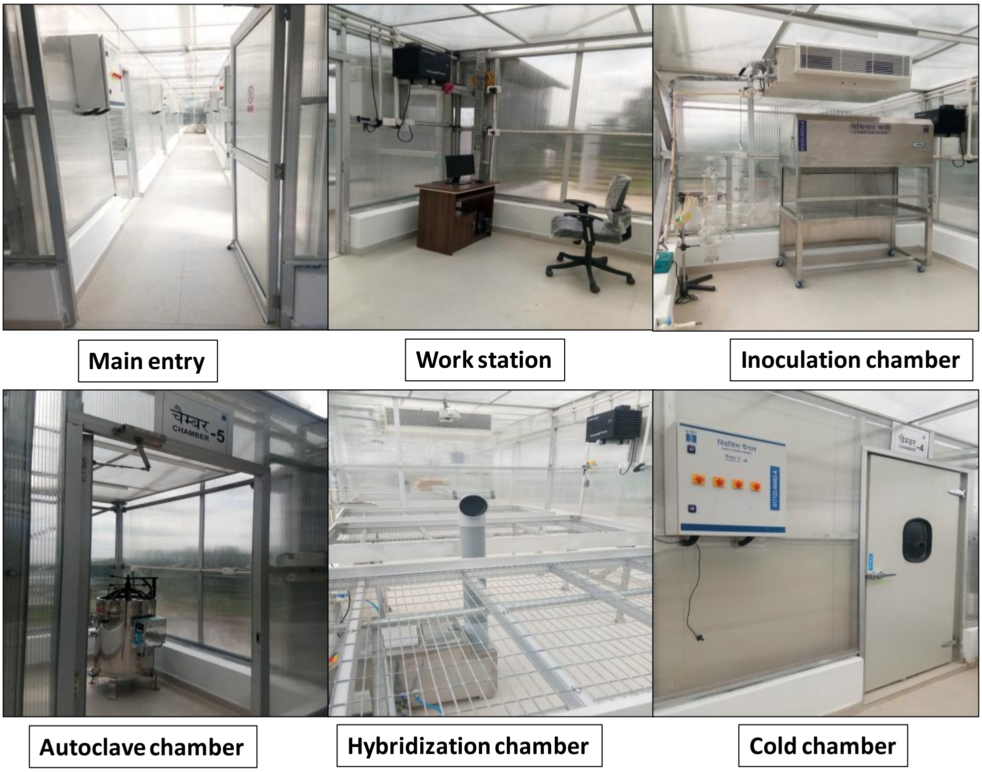

Figure 3. Different chambers in Phytotron facility

Different Sections of Phytotron

1. Chamber 1 (Work Station)

The very first chamber of Phytotron is established to carry out research work related to microscopy as one microscope is installed, chilling treatment or storage of chemicals in low temperature as one refrigerator is installed in this chamber. All work regarding documentation of the phytotron is also processed via this temperature controlled chamber. Microscope utilized for the investigation of the inner structure of plants (cell organelles, cell size etc.), flowers and other plant structures, microorganisms, pathogen infestation, disease symptoms, microspores etc.

2. Chamber 2 (Plant Growth Room)

This walk-in controlled-environment room provides growing areas of 15 m^2. Standard plant growth chamber maintain wide range of temperature, humidity, light intensity patterns and CO_2 that is essential for plant growth. The most important aspect is maintenance of high sterility in this room. It is cut off from the outside air completely and is completed isolated. It should be cleaned regularly so as to avoid any sort of dust deposition. For placing cultures, this room should have enough wooden or iron racks. Each rack should have tube lights and bulbs for providing the required photoperiod. Culture room should attach with a dark room for housing liquid cultures and other cultures requiring dark conditions. Walk-in Plant Growth Chamber will be used for research work to maintain controlled growing conditions of temperature, light and humidity in a 24 hours cycle and also to maintain CO_2 level. Walk-in Plant Growth Chambers will also allow the access to the world's most climatic conditions while eliminating the variability found in nature. Photoperiod room temperatures can be maintained at any point between 10 and 35°C. Photoperiods of all durations can be provided, including the natural progression of day length at any latitude. Each room is equipped with cool-white fluorescent and incandescent lamps that can be programmed separately, together, or alternately on any cycle from a few seconds per minute to continuous light. Phytochrome photo equilibrium can be altered by filters and different kinds of fluorescent and incandescent lamps.

3. Chamber 3 (Hybridization room for *Rabi* crops)

This chamber serves the purpose for growing and allowing the off season crossing between the desired genotypes/varieties of crops belonging especially to Rabi season thus saving time and energy needed for the development of improved varieties. Crops such as wheat, oat, barley, forage grasses (Tall Fescue, Rye Grass, clovers, berseem etc.), chickpea, lentil, mustard, crucifers, cole crops, peas, potato etc. can be grown and suitable hybrids can be developed by maintaining the appropriate temperature i.e. upto 18-22°C in the chamber to fasten the breeding cycle and to attain the generations in short period of time.

4. Chamber 4 (Cold room)

This chamber is completely refrigerated and equipped with cooling system and humidity control system. The winter crops require chilling temperatures that should be satisfied in order to initiate growth and flowering. They undergo a cycle of dormancy requirement that inhibits growth until exposure to low winter temperatures (chilling), prior to spring bud break. The duration of the dormancy period minimizes subsequently low temperature damage to flowers by delaying bud break and flowering. Also, lack of effective chilling duringwinters in tropical and sub-tropical areas result in prolonged dormancy leading to poor blooming, strong apical dominance and unsynchronized growth patterns and, consequently low yields (Cook and Jacobs, 1999). Stratification is a method of handling dormant seed in which the imbibed seeds are subjected to a period of chilling to ripen the embryo in alternate layers of sand or soil for a specific period. It is also known as moist chilling. Temperate species displaying epicotyl dormancy or under developed embryo a warm stratification of several months followed by a moist chilling stratification is required. Outdoor stratification leads to destruction of planting materials due to improper aeration, freezing, drying, or by attack of non-insect pests. Therefore, alternative refrigerated rooms are required for chilling treatment and overcome the outdoor limitations.

5. Chamber 5 (Autoclave room)

This chamber has disinfection and sterilization facilities which are equipped with autoclave like equipment along with ventilation system.The autoclave operates on the moist heat sterilization concept. For equipment sterilization, the high pressure within the chamber raises the boiling point of water. The greater pressure also guarantees that heat gets into the deeper regions of the equipment quickly. It operates at high temperature and the moisture in steamcauses microbial proteins to coagulate, resulting in permanent loss of activity and function in order to kill unwanted microorganisms and spores. It is mainly used for decontaminating and sterilizing the medias, test tubes, petri-dishes and other lab instruments.

6. Chamber 6 (Inoculation room)

Tissue culture techniques can be successfully carried out in a clean and dry atmosphere with protection against air-borne micro-organisms. This room should be perfectly sterilized, clean and dust free for routine transfer and manipulation work and it should have an arrangement of UV lights on the walls. The inoculation chamber consists of the Laminar airflow cabinets. It is the most common accessory used as a bench for aseptic manipulation/transfer of the plant materials in culture vessels. One or more laminar airflow cabinets can be housed in such inoculation rooms.

7. Chamber 7 and Chamber 8 (Hybridization room for *Kharif* crops)

These chambers are mainly meant for the purpose of speeding up the breeding process of various *Kharif* crops like rice, maize, sorghum, pearl millet, soybean, pulses, vegetables like okra, chilli, capsicum, tomato, brinjal, cucurbits etc. Chamber 7 consist of variation for controlled temperature at 28±3°C whereas chamber 8 has facility of maintaining temperature conditions upto 25±3°C, depending upon the requirement of the crop.

WORKING PRINCIPLES OF PHYTOTRON FACILITY

The environment has a significant impact on plant development and dispersal. A plant's development or dispersion are limited by any environmental element that is less than optimum. In deserts, for example, only plants suited to low water availability may survive. Environmental stress causes most plant issues, either directly or indirectly. In some circumstances, bad environmental conditions (such as a lack of water) directly harm a plant. Environmental stress can also weaken a plant, making it more vulnerable to disease or insect assault. Light, temperature, water, humidity and nutrition are all elements that influence plant growth. It's critical to comprehend how these variables influence plant growth and development.

1. Light

The colour or wavelength of light is referred to as light quality. The sun emits wavelengths ranging from 280 to 2800 nanometers (97 percent of total spectral distribution). Ultraviolet (100-380 nm), visible light (380-780 nm) and infrared are the three categories (700-3000 nm). Plant growth is influenced by three main qualities of light: amount, quality and duration.

Quantity

The intensity, or concentration, of sunlight is referred to as light quantity. It changes according to the seasons. In summer, there is the most light, and in winter, it is the least. The more sunlight a plant receives, the better its capacity for photosynthesis-based food production. Different plant development patterns can be achieved by manipulating light amount. Increase light by using reflective materials, a white background, or supplementary lighting to surround plants. Reduce it by using cheesecloth or woven shade cloths to shade plants. The lowest wavelengths have the maximum energy; ultraviolet has more energy than red.

Humans can detect the wavelengths between 380 and 770 nm, which are known as visible light (Feng et al. 2019).

Quality

The colour (wavelength) of light is referred to as light quality. Sunlight contains all wavelengths and can be divided into bands of red, orange, yellow, green, blue, indigo, and violet using a prism. Plants absorb blue and red light, which has the greatest impact on their growth. Vegetative (leaf) growth is significantly aided by blue light. When coupled with blue light, red light promotes flowering. Plants appear green to us because they reflect green light rather than absorb it. When it comes to controlling plant development, knowing which light source to utilize is crucial. Fluorescent (cool white) light, for example, has a high blue wavelength. It promotes leafy development and is ideal for seedling propagation. Incandescent light is bright red or orange, but it creates too much heat to be a useful source of light for plants. Fluorescent growlights use a combination of red and blue wavelengths to mimic sunlight, although they are expensive and often no better than conventional fluorescent lights (Yavari et al. 2021).

Different color of light and their impact on plant growth

1. **Ultraviolet Light:** Ultraviolet light causes DNA damage, reduces photosynthesis rate, flowering and pollination decrease, and seed development is affected. Ultraviolet A (a subcategory of ultraviolet light) can cause plant elongation.
2. **Blue Light:** It corresponds to one of the absorption peaks; therefore, the photosynthetic process is more efficient when there is blue light. Blue light is responsible for vegetative and leaf growth and is important for seedlings and young plants because it helps reduce plant stretching.
3. **Red Light:** This is the other peak of light absorption by the leaves. Phytochrome (a photoreceptor) within the leaves is more sensitive and responds

to red light. This light is important in the regulation of flowering and fruiting. It also helps increase stem diameter and promotes branching.

4. **Far Red Light:** This light can cause plant elongation and trigger flowering in long-day plants.

5. **Far Red Light and Ratio:** Plant elongation results when this ratio is low. In other words, plants are more exposed to far red than red. In nature, we see this phenomenon when plants are shaded by neighboring plants; the shaded plants receive a higher ratio of far red light and tend to grow taller to reach more light. This can become a problem with greenhouse crops that are shaded by overhead baskets or are planted too close together.

Sources available in Phytotron

In phytotron facility, there are two types of light emitting tools available that allow different types of light to the plants to activate physiological activities to reduce maturation time and helps to accelerate generation advancement in plant breeding materials and develop varieties rapidly. These systems can also enhance genetic gain due to taken more than one generation of plant breeding materials per year.

1. **Fluorescent lamps:** They generate most of their light in the blue, green and red spectrum, with the highest light level coming from the blue spectrum.

2. **High pressure sodium lamp:** The highest peak is green closely followed by red.

Duration

The length of time a plant is exposed to light is referred to as its photoperiod. Flowering is controlled by photoperiod in many plants. Initially, scientists believed that the length of the light period caused blooming and other reactions in plants. Plants are classified as short-day or long-day based on the conditions in which they blossom. We now know that the amount of unbroken darkness, not the length of the light phase, is crucial for floral growth. Depending on their reaction to the length of light or darkness, plants are classed as short-day (long-night), long-day (short-night), or day neutral. Short-day plants only blossom when the day is less than 12 hours long. Many spring- and fall-flowering plants, such as chrysanthemum and Christmas cactus, are in this category. In contrast, long-day plants form flowers only when day length exceeds 12 hours. Most summer flowering plants, as well as many vegetables, are in this category. Day-neutral plants form flowers regardless of day length. Examples are tomato, maize, cucumber and some strawberry cultivars (Gardner et al. 2006).

2. Temperature

Temperature influences most plant processes, including photosynthesis, transpiration, respiration, germination and flowering. As temperature increases (up to a point), photosynthesis, transpiration and respiration increase. When combined with day-length, temperature also affects the change from vegetative (leafy) to reproductive (flowering) growth. Depending on the situation and the specific plant, the effect of temperature can either speed up or slow down this transition.

Germination

Germination temperature varies depending on the species. Warm-season crops (e.g., tomato, petunia, and lobelia) germinate best at 65° to 75°F, whereas cool-season crops (e.g., spinach, radish and lettuce) germinate best at 55° to 65°F. Flowering Horticulturists sometimes influence blooming by combining temperature and day duration. A Christmas cactus, for example, produces blossoms as a result of short days and cold temperatures (Figure 26). Place a Christmas cactus in a room with more than 12 hours of darkness each day and a temperature of 50° to 55°F until flower buds form to encourage it to bloom. Cool-season crops will flower if the weather is hot and the days are long (bolt).

However, if temperature is too cool, fruit will not set on warm-season crops such as tomato.

Crop quality

Low temperature reduces energy use and increase sugar storage. Adverse temperatures, however, cause stunted growth and poor-quality vegetables.

Photosynthesis and respiration

Thermoperiod refers to daily temperature change. Plants grow best when daytime temperature is about 10 to 15 degrees higher than nighttime temperature. Under these conditions, plants photosynthesize and respire during optimum daytime temperatures and then restrict respiration at night. Temperatures higher than requiredenhance respiration rate than the rate of photosynthesis. Thus, photosynthates are used faster than they are produced. For growth to occur, photosynthesis must be greater than respiration. Daytime temperatures that are too low often produce poor growth by slowing down photosynthesis. The result is reduced yield.

Breaking dormancy

Some plants that grow in cold regions need a certain number of days of low temperature. Knowing the period of low temperature required by a plant, if any, is essential in getting it to grow to its potential. The cold temperature allows the bulbs to mature in bulbous crops such as saffron and kalazeera. When transferred to a greenhouse in midwinter, they begin to grow and flowers are ready to cut in 3 to 4 weeks.

Hardiness

Plants are classified as hardy or non-hardy depending on their ability to withstand cold temperatures. Hardy plants are those that are adapted to the cold temperatures of their growing environment. Woody plants in the temperate zone have very sophisticated means for sensing the progression from fall to winter. Decreasing day length and temperature trigger hormonal changes that cause leaves to stop photosynthesizing and to ship nutrients to twigs, buds, stems and roots. An abscission layer forms where each petiole joins a stem and the leaves eventually fall off. Changes within the trunk and stem tissues over a relatively short period of time "freeze-proof" the plant.

Winter injury to hardy plants generally occurs when temperature drop too quickly in the fall before a plant has progressed to full dormancy. In other cases, a plant may break dormancy in mid- or late winter if the weather is unseasonably warm. If a sudden, severe cold snap follows the warm spell, otherwise hardy plants can be seriously damaged. It is worth noting that the tops of hardy plants are much more cold-tolerant than the roots. Plants that normally are hardy to 10°F may be killed if they are in containers and the roots are exposed to 20°F.

Winter injury also may occur because of desiccation of plant tissues. People often forget that plants need water even during winter. When the soil is frozen, water movement into a plant is severely restricted. On a windy winter day, broadleaf evergreens can become water deficient in a few minutes and the leaves or needles then turn brown. To minimize the risk of this type of injury, make sure your plants go into the winter well watered.

3. Water and Humidity

Most growing plants contain about 90 percent water.

Water plays many roles in plants. It is:

1. A primary component in photosynthesis and respiration.
2. Responsible for turgor pressure in cells.
3. Responsible for the fullness of plant tissue.
4. Turgor is needed to maintain cell shape and ensure cell growth.
5. A solvent for minerals and carbohydrates moving through the plant.

6. Responsible for cooling leaves as it evaporates from leaf tissue during transpiration.
7. A regulator of stomatal opening and closing, thus controlling transpiration and, to some degree, photosynthesis.
8. The source of pressure to move roots through the soil.
9. The medium in which most biochemical reactions take place.

Relative humidity

Relative humidity is the ratio of water vapor in the air to the amount of water the air could hold at the current temperature and pressure. Warm air can hold more water vapor than cold air. Water vapor moves from an area of high relative humidity to one of low relative humidity. The greater the difference in humidity, the faster water moves. This factor is important because the rate of water movement directly affects a plant's transpiration rate. The relative humidity in the air spaces between leaf cells approaches 100 percent. When a stoma opens, water vapor inside the leaf rushes out into the surrounding air, and a bubble of high humidity forms around the stoma. By saturating this small area of air, the bubble reduces the difference in relative humidity between the air spaces within the leaf and the air adjacent to the leaf. As a result, transpiration slows down.

If wind blows the humidity bubble away, however, transpiration increases. Thus, transpiration usually is at its peak on hot, dry, windy days. On the other hand, transpiration generally is quite slow when temperature is cool, humidity is high, and there is no wind. Hot, dry conditions generally occur during the summer, which partially explains why plants wilt quickly in the summer. If a constant supply of water is not available to be absorbed by the roots and moved to the leaves, turgor pressure is lost and leaves go limp.

Plant Nutrition

Plant nutrition often is confused with fertilization. Plant nutrition refers to a plant's need for and use of basic chemical elements. Fertilization is the term used when these materials are added to the environment around a plant. A lot must happen before a chemical element in a fertilizer can be used by a plant. Plants need 17 elements for normal growth. Three of them, carbon, hydrogen and oxygen are found in air and water. The rest are found in the soil. Six soil elements are called macronutrients because they are used in relatively large amounts by plants. They are nitrogen, potassium, magnesium, calcium, phosphorus, and sulfur. Most of the nutrients a plant needs are dissolved in water and then absorbed by its roots. In fact, 98 percent are absorbed from the soil-water solution, and only about 2 percent are actually extracted from soil particles.

Fertilizers

Fertilizers are materials containing plant nutrients that are added to the environment around a plant. Generally, they are added to the water or soil, but some can be sprayed on leaves. This method is called foliar fertilization. It should be done carefully with a dilute solution, because a high fertilizer concentration can injure leaf cells. The nutrient, however, does need to pass through the thin layer of wax (cutin) on the leaf surface. Plants produce their own food from water, carbon dioxide and solar energy through photosynthesis. This food (sugars and carbohydrates) is combined with plant nutrients to produce proteins, enzymes, vitamins, and other elements essential to growth.

Nutrient absorption

Anything that reduces or stops sugar production in leaves can lower nutrient absorption. Thus, if a plant is under stress because of low light or extreme temperature, nutrient deficiency may develop. A plant's developmental stage or rate of growth also may affect the amount of nutrients absorbed. Many plants have a rest (dormant) period during part of the year. During this time, few nutrients are absorbed. Plants also absorb different nutrients as flower buds begin to develop than they do during periods of rapid vegetative growth (Morgan and Connolly 2020).

In working principle, Phytotron permit to maintain precise control over environmental conditions (e.g. soil type, air temperature, relative humidity, light levels, carbon dioxide (CO_2) and ozone (O_3) concentration) and the organisms under study (e.g. mixtures of plant species and their spatial arrangement). Such a degree of rigor and control is virtually impossible to achieve under natural field conditions. Phytotron have been exploited to provide controlled and reproducible conditions for several types of studies in plant physiology, phenology, plant biochemistry, agronomy, ecology, plant morphometry, food quality and plant pathology.

OPERATIONAL PROCEDURES IN PHYTOTRON

All supplies, equipment, plants and substrates enter and leave the Phytotron via the receiving room. Most items are fumigated before transfer to the Phytotron interior however sterile cultures, research material and analytical instrumentation can be exempted.

Soils and other non-Phytotron substrates must be sterilized so only the substrate container is involved in the fumigation process. It is to everyone's advantage to observe carefully the clean-up procedures and to attend first to Phytotron work before visiting other greenhouses or field locations.

Responsibility of the Investigator

The project leader is expected to use clean seeds, free of latent diseases and insect problems. The investigator is responsible for planting the seeds and will arrange for all experimental treatments and special watering programs. Well-trained personnel can be hired through the Phytotron Incharge as hourly assistants to aid in initiating experiments, assigning or administering experimental treatments, and taking data.

The project leader is expected to assume responsibility for the safety training for all members of the research group.

Special care should be taken to identify the desired watering schedule. Since the investigator establishes the routine of the research program, it seems reasonable to expect the schedule to be followed.

Role of the Phytotron Staff

The Phytotron staff sets up and maintains the environmental conditions. Any suspicion of an off-normal situation should be reported immediately to Phytotron Staff. The Phytotron staffs are responsible for day to day operation and maintenance of greenhouse, growth chambers, growth rooms and all associated equipment.Staffs have to maintain financial records for Phytotron expenditures, continuously planning to improve Phytotron operations and associated equipment, will be on standby to respond to alarms due to equipment failure or service interruptions with critical utilities.

Phytotron Operating Schedule

With few exceptions, environmental conditions are established early in the week and allowed to proceed for a 24 hour period before experiments are initiated. Once the experiment is started, the chambers are not to be opened by anyone, including the investigator, when the dark-condition, red-light indicator is on. Only by following this rule can everyone be assured of photoperiod integrity.

Visitors

Members of the Phytotron staff are always available to discuss the facility with visitors. Phytotron users also may conduct visits through the facility. The visitors should be asked to record their names and organizations in the guest book located in the buffer room.

General Guidelines for studies conducted in Phytotron facility

- ✓ Keeping work areas orderly for the duration of the experiment; particular attention must be given to neatness and sanitation
- ✓ Technical staffs carry out the daily care of the plant materials, controlled-environment rooms and building.
- ✓ The use of dangerous products within the facility must be carefully managed to prevent risk to personnel or other users, damage to equipment and to avoid disruption of neighboring experiments.
- ✓ The use of products that would potentially have residual effects, thus impacting subsequent users of the equipment, is not permitted.
- ✓ The care of experimental organisms and materials within the facility is the responsibility of the research user. This includes the proper watering, fertilizing and pruning of plants; handling, containment and feeding of insects, growth, handling and containment of bacterial, viral or fungal organisms.

- ✓ Technical staffs have the responsibility of monitoring experimental populations for the presence of problems, applying preventative and prescriptive treatments according to a set of well established protocols, and communicating progress with the research users.
- ✓ The Phytotron staff is responsible for caring for the facility and its experimental contents during emergency situations (power & water shutdowns, unusual weather phenomena). All possible resources will be mobilized to permit the survival of organisms and satisfactory progression of experiments.
- ✓ The primary users also share in the responsibility of training and supervising their own students and staff members to assure that Phytotron rules as well as sound scientific procedures are understood and being followed.

SALIENT ACHEIVEMENTS

1. Research to be done and being undertaken in the University

Oat haploids production through Oat × Maize hybridization

a. Off-season hybridization programme

When environmental conditions are not congenial for crop of interest, one may go for off-season crossing by creating suitable artificial conditions. The chamber 3 in the phytotron is mainly developed for the breeding of *rabi* crops whereas chamber 7 and 8 are meant for *kharif* crops. This will aid in early completion of breeding cycles, with 2 or more than 2 generations per year for accelerating the varietal development programmes and in turn which is an asset for rapid generation advancement thus saving half of the time and energy than required for conventional approaches. The research has been undertaken till date and is still going on in many crops like wheat, oat, rice, mash, moong, soybean and buckwheat etc.The work is also going on for development of mapping populations in the shortest possible period needed for targeting novel genes (drought, cold, heat, frost tolerance, rusts, blight and powdery mildew resistance etc.) in different field crops.

Generation advancement in wheat and rice

b. Acceleration breeding programme in cereals, vegetables and oilseeds

When haploid undergo chromosome doubling, a genotype known as a doubled haploid (DH) is generated. Facilities for tissue culture like meristem culture and embryo culture have been established for haploid production and development of disease free plants within phytotron. Wheat haploid and DH plants by *Imperata cylindrica* and maize-mediated approaches of chromosome elimination have been developed in phytotron. DH breeding speeds up mapping population and cultivar development. The development of doubled haploid plants has a direct impact on breeding since it achieves homozygosity in segregating populations in a single generation. This allows steady line selection to begin much earlier. Currently DH in wheat is developed successfully whereas works in oat and vegetable crops are still in progress. Screening of germplasm under diverse environments is created artificially and are conserved & maintained in order to introduce or introgress variability and gene(s) imparting tolerance to diverse biotic and abiotic challenges into breeding programmes.

c. Breaking the dormancy of potential crops like kalazeera and saffron

Saffron (*Crocus sativus* L.) and Kala zeera (*Bunium persicum* Boiss) are temperate region crops and are loaded with many beneficial nutrients. The gap between demand and supply is increasing due to urbanization, unsustainable exploitation and biotic and abiotic stresses lead to loss of yield and diminishing of natural populations, so an immediate production is urgently required. Recently, some regions of HP also have been found congenial for its cultivation. The corms and bulbs of such crops are now developed through micropropagation technique under controlled condition. The work has been going on in the established phytotron to manipulate dormancy by cold treatments and develop the propagating material (microcorms) and their mass multiplication in short time span.

Breaking the Barrier of potential crops like kala zeera and saffron cultivation

2. Publications and awards

Kumar S, Sood VK, Sanadya SK, Sharma G and Kaushal R. 2022.Introgression of quality and yield traits from wild *Avena* species to cultivated oat (*Avena sativa* L.) and identification of introgressed alien chromatin using morphological and molecular techniques. 1st International Symposium held at IIWBR, Karnal on January 18-20, 2022. pp: 18-19

Singh, K, Chaudhary, HK, Manjoj, NV and Verma S. 2022. Inheritance studies of yellow rust resistance in bread wheat genotypes for *Yr5* gene. *Biological Forum- An International Journal* 14(3): 777-779.

Verma S, Chaudhary HK, Kumar N and Singh K. 2022.Screening of bread wheat genotypes for strip rust and powdery mildew resistance. 1st International Symposium held at IIWBR, Karnal on January 18-20, 2022. pp: 146

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Phytotron facility demonstration for students and trainees of various Universities

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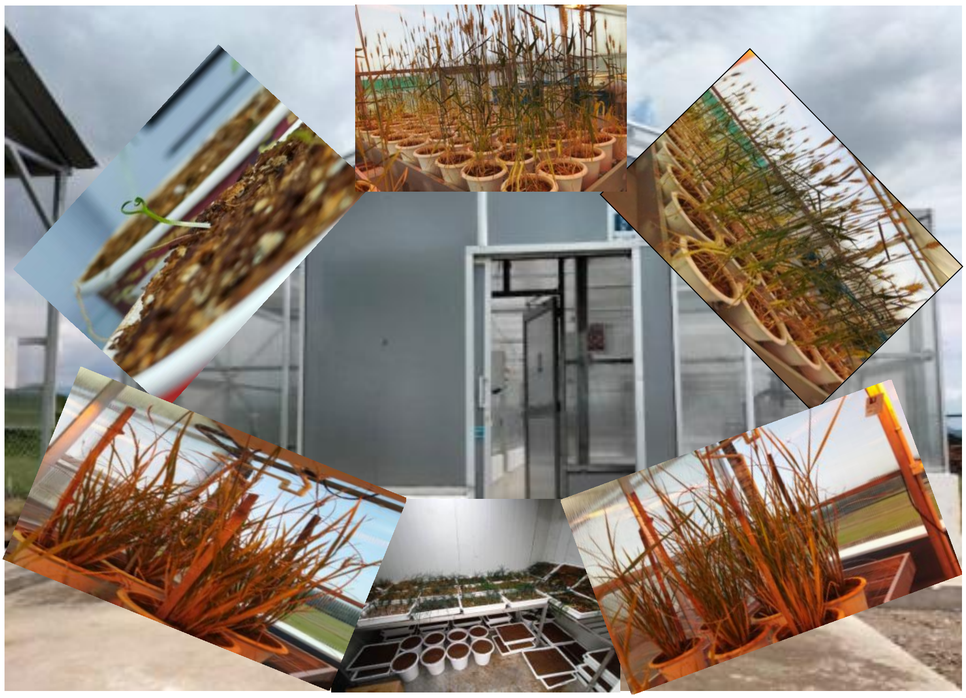
Yavari N, Tripathi R, Wu BS, MacPherson S, Singh J and Lefsrud M. 2021. The effect of light quality on plant physiology, photosynthetic, and stress response in *Arabidopsis thaliana* leaves. *PLOS ONE* 16(3): e0247380.

NOTES



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