RP Current Trends In Applied Sciences
Vol. 2, No. 1 (January – March 2023) pp. 22–29
e-ISSN: 2583-7486

RESEARCH PLATEAU PUBLISHERS

Cite this article: D. Rani, A review on pollen storage and the factors affecting its viability, *RP Cur. Tr. Appl. Sci.* **2** (2023) 22–29.

Review Article

A review on pollen storage and the factors affecting its viability

Deepika Rani*
Department of Botany, MNS Government College, Bhiwani – 127021, Haryana, India
*Corresponding author, E-mail: dippi6953@gmail.com

ARTICLE HISTORY
Received: 22 Nov. 2022
Revised: 30 January 2023
Accepted: 31 January 2023
Published online: 05 February 2023

KEYWORDS
Pollen longevity; storage; cryopreservation

ABSTRACT
Breeding programmes, genetic preservation, artificial pollination, and self-incompatibility all benefit from pollen storage. Pollen longevity varies tremendously depending on the type of plant and how it is stored. Today, a variety of methods are used to keep pollen viable while it is being stored. In contrast to trinucleate pollen, mostly binucleate pollen can be kept in storage for extended periods of time without losing vitality. Compared to high temperature, pollen that had been held at a low temperature had a superior ability to germinate. The pollen viability is influenced by a variety of factors. This study emphasises pollen storage conditions and variables influencing pollen vitality.

1. Introduction

Because viable pollen is essential for effective sexual plant reproduction, pollen represents an important step in the life cycle of plants. Viability and vigour are used to judge the pollen's quality. After suitable pollination, the pollen's viability refers to its capacity to transport functional sperm cells to the embryo sac [1]. Small amounts of reserve food, typically in the form of starch or sugars, lipids, and proteins, are present in mature pollen grains, which are relatively dormant at this stage.

Pollen that is still viable and mature has a non-vacuolate, granular cytoplasm that is packed full of plastids, mitochondria, golgi-derived vesicles, lipid droplets, and a significant number of dormant dictyosomes [2]. Rapid and significant changes occur before and after germination. During pollen hydration at the time of germination, the pollen plasmalemma's permeability is changed [3]. The primary beginning factors for germination are the intake of water and the activation or production of enzymes. Long before tube growth is visible, germinating pollen is already respiring and metabolising exogenous sucrose. Rapid starch buildup in germination-ready pollen is accompanied by an initial high rate of respiration [4, 5]. Protein and nucleic acid synthesis occurs during the activation phase, prior to the development of the pollen tube [6–8].

Before the pollen tubes are produced, the pollen grain wall contains several enzymes that easily diffuse into the surrounding liquid [9]. Many of these are enzymes that hydrolyze and soften cell walls [10]. When pollen grains are placed in a germination media, free amino acids are quickly released [11].

In sugar solutions, pollen grains from several different species effectively germinate. The best and most popular source of carbon and energy for pollen is probably sucrose. It creates and sustains the ideal osmotic conditions for pollen germination and sustained pollen tube growth. Boron addition to the medium promotes pollen tube growth and germination. For plants to thrive and produce at their highest levels, the element of boron must be present in sufficient quantities. A lack of boron impairs pollen viability, pollen germination, and pollen tube growth as it affects the blooming and fruiting processes of plants [12].

Boron has a crucial role in the fertilisation of flowering plants because it participates in pollen germination and the production of style tubes. Boron controls the hydration of colloids, is linked to pollen membrane polyhydroxyl compounds, and helps create pectic materials for tube walls [13, 14]. For example, it is well known that removing boric acid from the culture medium frequently results in tube bursting [15]. Boron, which is provided in the form of boric acid, is also necessary for the in vitro culturing of pollen from the majority of species. The best in vitro pollen germination conditions for pistachio trees, according to Acar [14], were a 20% (w/v) sucrose medium, the hanging drop germination method, and a temperature of 25°C in complete darkness for 24 hours. The expanding pollen tube in nature is fed by the style, which also provides water, sugar, and amino acids. For the formation of pollen tubes in many species, boron and calcium are also necessary. Boron, which is present in stigmas and styles, aids in the intake of sugar and contributes to the synthesis of pectin in the pollen tube [16].

Auxins, gibberellins, and cytokinins are known to be present in quite high concentrations in the pollen grains of several species [17]. It is not surprising that exogenously added concentrations of plant growth factors to pollen fail to significantly boost pollen germination and tube growth [18]. One study revealed the impact of GA administration on pollen tube growth in vivo or in vitro [19]. For instance, GAs are also present in growing pollen after anthesis. GAs can either stimulate, hinder, or have no effect on pollen germination and

Copyright: © 2023 by the authors. Licensee Research Plateau Publishers, India
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

tube elongation in vitro, depending on the species being studied and the concentration utilised [20].

The wall of the pollen tube stretches as a result of many variables, including turgor pressure. Such stretching is only present in the tip region, where vesicles fuse with the plasmalemma to deposit new material. According to Kroeger et al. (2009), vesicles are discharged into the inverted cone as they advance along the cortex via the actin fringe. Diffusion may now be used to control vesicle mobility. Vesicles are drawn acropetally towards the apical plasma membrane by the process of exocytosis. There are no cell organelles like mitochondria, golgi bodies, ER, amyloplasts, or lipid structures in the non-vacuolated, agranular cytoplasm of the cap block, and expanding pollen tubes do not exhibit cytoplasmic streaming either.

Protein synthesis occurs concurrently with the relatively dormant pollen grain's activation during germination, followed by RNA synthesis. In pollen tubes, the developing tip region, which is devoid of ribosomes but teeming with smooth membranes that are vulnerable to RNase treatment, contains the highest concentration of RNA. For germination and early pollen tube expansion, pollen synthesised proteins are necessary [21]. The research of pollen tube development has benefited from the use of cycloheximide and actinomycin D [22–24]. Cycloheximide had no discernible effects on pollen germination and early tube growth in an in vitro experiment, according to Roberts et al. [25]. While Speranza et al. [26] observed that cycloheximide totally prevented kiwifruit (Actinidia deliciosa var. deliciosa) pollen germination. Actinomycin and cycloheximide, respectively, were discovered by Fernando etal. [23] to inhibit the RNA and protein synthesis during pollen germination and tube elongation in coniferous species. The current section analyses the most recent data on the factors contributing to pollen viability loss as well as the circumstances allowing for longer viability and storage times.

2. Storage of pollen

In order to create new and improved plant kinds that are more suited to human needs, horticulturists and plant breeders have long been interested in crossing varieties, species, and even genera. However, many of these attempts have been unsuccessful due to the barriers to crossability, such as the chosen parents flowering at different times or locations, the pollen grains failing to germinate on the stigma, the pollen tubes bursting in the style, the tubes failing to grow through the style, and the slow growth of the tubes preventing them from reaching the ovules prior to the flower's abscission. There is no question that pollen tubes occasionally enter the embryo sac, but sterility is the result of either the male gamete failing to fuse with the egg nucleus or the embryo and endosperm failing to develop properly or failing to develop at all. Utilising stored pollen is the most effective way to get over this time and space-based obstacle. Additionally, pollen storage eliminates the need to continuously grow the common cross-use lines. Additionally, it offers more adaptability for pollen experimentation.

Short-Term Storage in Organic Solvents

Pollen solvent storage gets over the issue of maintaining relative humidity and might be a practical method for delivering pollen cooling. Iwanami was the first to investigate the effectiveness of organic solvents in pollen preservation; in addition, the comparative effectiveness of various organic solvents in Camellia [28] has provided insight into future research on other species [29]. The effectiveness of various solvents for preserving pollen of some leguminous taxa has been thoroughly investigated [30], demonstrating the species-specific responses. Jain and Shivanna, 1990, have provided a more complete assessment of the effects of various solvents on the leaching of phospholipids [31] and their link to viability as a result of their implications on membrane integrity [32]. All of these studies also highlight how polar and non-polar solvents behave differently in terms of their ability to preserve materials; it was asserted that polar solvents like hexane and diethyl ether cause much less leaching of substrates like sugars, phospholipids, and amino acids than pollen stored in non-polar solvents.

Numerous structural and functional changes in the cell are quite irreversible, according to studies on the moisture content and the effects of dehydration and rehydration on pollen. As a result, many taxa's pollen viability could be prolonged for shorter periods of time through appropriate manipulations of the water content and humidity of the storage conditions. For a variety of plants, the relative effects of moisture content and storage temperature on the duration of storage are highly variable [33].

According to reports, viability is quite brief in several species of graminae with tri-nucleate pollen when grown naturally [34]. Even the circumstances for short-term storage are less efficient than in other angiosperm families. It has been demonstrated that wheat pollen that can last up to one to three hours in storage at 20°C can do the same at 4°C. Jones and Newell [35] estimated the pollen viability of maize to last up to 9 to 11 days at 4°C and 90% relative humidity; however, subsequent studies [36] found that this was reduced to six days with an additional 5–10% rise in relative humidity. By lowering the temperature to 4°C at equal humidity levels, the Secale cereale's 12-hour storage time at 17–21°C storage temperature could be extended to 4–7 days.

The vitality up to 14 days at 14°C was decreased to 4 days at 4°C in Pennisetum, on the other hand. In addition, when applied 3 weeks later, the seed set was only 8% as good as fresh pollen. Our laboratory's pollen research on pearl millet shown that pollen vitality could not be increased for more than 72 hours when stored at either 0°C or 4°C. However, research has shown that keeping the Papaver at 4°C in a dessicator can extend its viability by up to 200 days. The "pollen-dryer" appears to be quite effective in controlling temperature and relative humidity (RH) in short-term storage techniques as well as the moisture and relative humidity of the air to achieve rapid and uniform dehydration. Buitink et al. [38] discovered via paramagnetic resonance spectroscopy that molecular mobility is negatively associated with storage stability and that the mobility is governed by water content. These studies offer estimates for the ideal storage conditions, and the correlation shows that molecular mobility must be at least somewhat responsible for storage stability. However, research conducted by Cheng [39] and Pacini et al. [40] using methods such as spin probe, electron paramagnetic resonance (EPR), and saturation transfer paramagnetic resonance (ST-EPR) has

revealed that partially hydrated pollen is frequently lacking in mechanisms to preserve pollen viability, indicating that the ideal levels of moisture and humidity must be properly assessed for each taxon.

Long - Term Storage

Although storing pollen above 0°C lengthened its shelf life for a small number of species, the results are not always positive; graminaceous members have substantially less success. Additionally, the use of freezing and cryogenic settings has been successful in the investigations that have come after. The findings of the investigations seem to be influenced by more variables in the protocols being used, despite the relatively encouraging results with these approaches; these are complicated to a certain extent, demonstrating diverse reactions, and are detailed below.

Use of Freezing Temperatures

Under this type of preservation, pollen might be kept fresh for a long time by being exposed to temperatures between -10 and 34°C. The approach is better suitable for extending the longevity of bi-nucleate pollen with low water content than tri-nucleate ones, according to a vast amount of data from a wide number of species [41]. Most species' pollen viability was increased to last for noticeably longer periods, varying from a few months to occasionally almost three years. Recent investigations [42] have shown that annual soybean pollen retains viability for four months when exposed to - 20°C, however in vitro germination with wild relatives failed in contrast to nearly normal germination with preserved pollen from cultivars. Similar research on lilies [43] showed that stored pollen (at - 20°C) delayed germination by one hour compared to fresh pollen; it was assumed that some proteins would degrade during storage and be synthesised during the lag period; this was confirmed by research on the qualitative and quantitative differences in the proteins of fresh and stored pollen [43].

Freeze-Drying and Vacuum-Drying

Lyophilization is another name for this method of pollen preservation. Since the first reports of freezing and drying applications [44], it has emerged as a viable instrument for the secure storage of a variety of biological materials [45, 46]. Additionally, it has been improved to a suitable degree for the retention of pollen from numerous species. The pollen would first be exposed to rapid freezing temperatures (-60 to -80°C), then the water would be gradually removed under vacuum sublimation, according to the freeze-drying procedure. On the other hand, in the vacuum-drying process, the pollen is directly exposed to vacuum and cooling at the same time as the moisture is removed by evaporative cooling, and the first freezing stage of the freeze-drying method is not involved.

Despite the fact that the first publications on lyophilization date back to the first decades of the 20th century [48], King [47] assessed the effectiveness of this approach in several species. The temperature, humidity, and pressure conditions [49] as well as the pressure-raising chemicals utilised have an additional impact on the procedure. The length of storage is significantly influenced by the presence of inert gases (such helium and nitrogen) or simply vacuum in the pollen environment. Alfalfa pollen and ovaries had a longer lifespan than before, according to Hanson [50], and pollen from many other species had a similar improvement. The vitality of pea pollen has been successfully dried using vacuum and frozen [51]. Pollen from Douglas-fir trees that had been freeze-dried for a variety of time periods did not affect the seed set, germinability, or seedling vigour, showing that it may be kept for a long time and safely [52]. The influence of pre-freezing as well as freezing temperatures of various periods needs to be identified in order to improve the storage conditions of pollen for each taxon since water molecules sublime with little change in molecular structure using this method. The vitality of the undried and air-dried samples of pollen from Pinus monticola was increased by cold storage of the pollen before freeze drying [53]. The pollen's water content appears to be a factor in the rate of water removal, which was slower for pollen kept in the cold. According to Ching & Ching [53], air-drying pollen for four hours or lightly refrigerating it for a few weeks before freeze-drying it for 30 to 60 minutes is sufficient to remove free water and maintain better vitality. While researching the potential of freeze drying on the pollen of Lilium and maize, the influence of temperature, humidity, and pressure on storage ability has also been emphasised [54]. According to Barnabas and Kovacs [55], these techniques are more successful at achieving somewhat longer periods of viability for taxa with desiccation-tolerant pollen.

Cryopreservation

When the technique was originally used to the pollen of Antirhinum majus, it became clear that pollen could be kept in unchanged circumstances by exposure to extremely low temperatures (-70 to - 196°C). The method's promise is clear from the numerous instances in which it has been used since 1950 to preserve pollen from various species. The effectiveness of utilising liquid nitrogen to cryopreserve pollen from agronomic species has been confirmed in a variety of species [56, 57], including numerous crop species in the family Graminae. The practical utility of it was demonstrated by their effects on storage, germinability, and the pollen of Pyrus malus and Pyrus communis. In contrast to the pollen remaining viable for up to 1062 days in the case of Lycopersican esculentum when exposed to -190°C and up to 5 Years in the case of Vitis vinifera, the viability was increased in these plants to 1 to 2 years [58].

The technique might not work as well for all species, though. Only shorter preservation times have been reported, such as in Glycine max (21 days), Gossypium hirsutum (only 10 days), and Vicia faba (up to around one month). When exposed to -196°C, Solanum tuberosum, the vitality of stored pollen increased from 9 months to 24 months. In Capsicum, the cryogenic approach not only increased longevity but also conserved the pollen's ability to fertilise; in wheat, the germination powers of cryopreserved pollen were comparable to those of fresh pollen. Ichikawa et al.'s experiments on the pollen of other tree plants, like peach and pears, were conducted after the studies on the pollen of numerous tree species, including larch (Larix Ieptolepis), which subjected its pollen to extremely low temperatures. Both pollen from horticultural species like roses and pollen from maize have been successfully preserved using this technique. The

importance of such techniques has been correctly emphasised by Shivanna and Johri [60] in their book on cryopreservation techniques. In order to achieve the best outcomes, numerous studies have suggested that the water content must be decreased to a certain point before the pollen is exposed to such low temperatures [61, 62]. In order to successfully cryopreserve different taxa over a long period of time, it is necessary to determine the crucial moisture level for each species' pollen. This is because dehydrated pollen has less freezable water and could survive extended exposure to freezing temperatures.

The results of research using cryogenic technologies on the germination and tube growth of frozen pollen as well as their subsequent impacts on fertilisation and seed germination reveal negligible variations from the regular pollen. The fluorochromatic (FeR) technique to assess the quality of the stored pollen, as it has been further developed recently [63], has much potential to assess the further quality of the pollen stored using all these different approaches. Staining protocols like enzymatic fluorescence are readily useful for quickly determining pollen viability.

3. Pollen banks

The asynchrony of flowering between cultivars and their wild relatives, which prevents future cross-breeding, is a significant setback in plant breeding [64]. Pollen preservation is also necessary because many recent haploid breeding techniques and biotechnology experiments use it as a source of material to achieve gene expression of the transformed pollen cells. The need for "pollen banks" has emerged as a potential strategy for the conservation of genetic resources that is comparable to the preservation of sperm and embryos in the case of animals in veterinary sciences, which facilitates their further transpor As shown by King's comments [65] - "control over the supply of pollen, so that we might use it when and where convenient to ourselves" - the significance of it was anticipated already in the late 19th century. As a result, they give scientists and breeders the option to use the source materials regardless of time or location, and they also do away with the necessity for nurseries and greenhouses for plant growth. Furthermore, the prospect for their use in gene manipulation studies is made possible by the comparatively straightforward methods of their collection and preservation as well as their abundance in relatively pure form. In addition to these, several proteins exhibit persistent expression, pointing to their potential use in transgenic research on all higher plants. Future research may be focused on better understanding the mechanisms that cause pollen membrane damage, preserving the structural integrity of pollen as it relates to its longevity, and improving storage techniques to make the most of the various pollen preservation techniques currently in use.

4. Factors affecting viability

Numerous biotic and abiotic factors have an impact on pollen viability following. In addition, the low moisture content, more resistant cell wall, and other characteristics of the two-celled pollen reduce the loss of viability, which naturally have a higher life-span as compared to the tri-nucleate pollen. The longevity of pollen, as influenced by the bi-nucleate or trinucleate conditions at the time of dispersal [66] was attributed to various structural and physiological changes. The pollen membrane is extremely labile and active. The growth of microspores to produce viable pollen grains is further hampered by chromosomal instability in the tapetal cells, which leads to programmed cell death [67]. Recent investigations on B-chromosomes, knobbed chromosomes, and NOR in maize have established this. Furthermore, a good deal of understanding existed on the relationship between the vegetative cell membrane and viability. The direct influence of phosopholipid membrane components on pollen viability suggests that structural changes to the pollen occur as a result of viability loss. Furthermore, during pollen water loss, the actin cytoskeletal components are probably going to be disturbed. It is claimed that this phase transition is responsible for the imbibitional damage in dry pollen, which could be remedied by gradually hydrating pollen or increasing temperature to effect transition to the more advantageous liquid crystalline phase. Fourier transform infrared spectroscopy (FTIR) studies of pollen [68, 69] are very helpful to reveal the changes in the molecular structures (if the membranes). All of these research demonstrate the necessity of creating methods that enhance pollen viability while maintaining the correct integrity of the membrane components.

Effect of Humidity

The response to high or low humidity varies by species and is typically correlated with the pollen's inherent level of hydration at the time of dehiscence [70]. Most species' pollen has very little water in it, although certain pollen grains have a rather high water content. For instance, the dehiscence of the pollen of the Gramineae contains more than 30% water [71]. These pollen grains are more metabolically active in addition to having higher amounts of moisture, which enables quick pollen tube extrusion [72]. Loss of water is thought to cause irreversible alterations in the pollen membranes of desiccation-intolerant species. Pollen grains from different species have evolved adaptations to reduce water loss to the environment. These could include modifications to the pollen wall's structural integrity or the presence of sucrose in the pollen cytoplasm to prevent membrane damage and water loss.

According to in vitro germination tests on the treated pollen grains, maize pollen grains practically lose all vitality after 3 hours when exposed to the atmosphere's drying conditions [73, 74]. Pollen gradually lost water as a result of exposure to the environment, and at water content values of 30% and lower, germination was significantly impeded [75]. The maize pollen grains changed throughout the experiment from being spherical and white to collapsing and yellow, demonstrating the evolution of the drying process.

The type of carbohydrates that are kept in the cytoplasm has been hypothesised to be related to the differences across species in viability loss when exposure to drying settings [86]. For instance, Pennisetum typhoides contains 14% sucrose and lives far longer than maize, which only contains 5% sucrose and swiftly loses viability due to dehydration [77]. In most species, loss of pollen viability results from a lack of water, but in some circumstances, pollen may also suffer from the availability of water in the form of rain. It has been demonstrated that water causes pollen to rupture, as in the case of cotton [78]. This phenomenon not only has a significant

impact on the development of bolls, but it also offers the potential to cause male sterility in plants that are in bloom.

At 17–22 oC, Pfundt [79] examined the effects of 0, 30, 60, and 90% relative humidity (RH) on the vitality of pollen from 140 species. It is clear from the observations that low relative humidities (0–30%) were the conditions that produced the longest lifespans. Apple, pear, grape, and plum lifespan of roughly a year was noted by Manaresi [80]. A number of taxa's pollen storage information under regulated temperature and humidity were listed by King [81]. The pollen of the gramineae is incredibly short-lived in comparison to other plants' pollen, which has a relatively long viability, and it is obviously damaging in the range of humidity (0-40%) that is beneficial to most other pollens. Pfeiffer [82] asserts that there is a close relationship between the lifetime of pollen and the amount of moisture in the air, with maxima and minima of various pollens at various humidity levels.

Effect of Temperature

In general, mature pollen grains may withstand temperature stress after dehiscence. Mild heat stress following dehiscence did impair fruit set in the tomato plant, but the differences from controls were not statistically significant [83]. Brassica pollen experiments showed that germination continued even after being exposed to 60°C for 4 hours; additionally, if the pollen was prehydrated in humid air prior to germination, they even exhibited germination after 24 hours at 45°C [84]. However, compared to controls, germination rates and pollen tube lengths were substantially lower. Pollination with pollen that had been heated to 75 or 60 degrees for 24 hours reduced seed set, while pollination with any of the other samples resulted in normal seed set.

Depending on the species, both heat and cold during pollen production might have a deleterious impact on pollen viability. It has been shown that nighttime temperatures below 10°C reduce pollen viability to 50% of controls on mango trees, while cold spells are linked to lower fruit set. The best conditions for tomato fruit set have been reported to be between 20 and 25°C. Unexpectedly, increasing the temperature to 29°C significantly decreased the amount of fruits that formed and the seed set. According to measurements of the starch and soluble sugars in tomato anthers and pollen that had undergone mild heat stress, the pollen grains did not exhibit the temporal increase in starch concentrations.

Short bouts of heat stress during the pollen development in Arachis hypogaea led to a decrease in fruit set, pollen yield, and pollen viability. The pollen viability of the heat-sensitive genotype of Phaseolus vulgaris decreased from 80% to below 10% after 10 days of heat treatment when compared to the heat-tolerant genotype during development. However, even after 24 days of stress, the heat-tolerant genotype continued to generate 60% viable pollen [85].

UV-B Radiation

Since it became known that a decrease in atmospheric ozone could result in an increase in UV-B radiation in the past 25 years [86], studies on the impact of UV-B radiation on plant growth and development have primarily been conducted. Since UV-B radiation (280–320 nm) is typically present in sunlight that reaches the earth's surface, it is likely that plants have defence systems in place to shield them from UV-B's potentially harmful effects. However, these defences might not be enough if radiation levels rise as a result of ozone-depletion. DNA, proteins, and lipids may be damaged by UV-B rays on a cellular level. Damaged proteins and lipids can be partially replaced, and DNA damage can be repaired. Additionally, when UV radiation targets membranes, some transport systems are disturbed, which in turn may upset cellular functions [87]. Increased UV-B radiation may slow plant development overall, but reactions vary between species and even within cultivars. UV-B radiation may influence flowering time and flower production in relation to reproduction. Flavonoids found in the pollen wall may mitigate any potential harm caused by UV-B radiation to the pollen during its transit from the anther to the stigma. Additionally, the interior pollen grains may be shielded from radiation damage by the dissemination of pollen that has been aggregated in some fashion [88].

Physiological and Biochemical Changes

A few of the substrates contained in the pollen and their subsequent metabolism are also significant factors in the loss of viability in some species, in addition to the pollen's level of hydration. Nielsen [1989] documented the impact of vitamins and how they affect survivability. Johri and Vasil's reviews [18] merely point out the physiological alterations in the pollen that account for viability. The water concentration of the pollen, as demonstrated by Buitink et al. [90], regulates vitality. However, there are differences in the physiological activities of the water in these two types of pollen based on the physical behaviour of the water in desiccation-sensitive and resistant pollen, which is not very varied. Wilson et al. [91] discovered a novel method for analysing anhydrobiosis and found that the dry pollen had a slower rate of metabolism.

However, rather than the slower rates of metabolism, the difference in viability in many plant species was related to the lack of metabolites. Changes in the endogenous levels of organic acids and carbohydrates also represent the physiological involvement in the loss of viability, and this role has been further discussed in their book [92]. Recent research has focused on the significance of carbohydrate metabolism and a unique pathway that operates in pollen/microspores. Glucose and fructose concentrations gradually decrease from one wall to fluid and from fluid to pollen, and any modification to the pollen that prevents this kind of flow into the pollen renders it inviable. In contrast, heat stress during pollen maturation in the anthers alters carbohydrate metabolism and lowers the sugar concentration in the pollen, resulting in an accumulation in the locular fluid and a reduction in pollen viability in tomato, as reported by Rao et al. [84]. In this situation, Wang and Song's [93] histochemical staining is very helpful for quickly and accurately determining the starch and lipid levels. It is more plausible that a lack of respiratory substrates causes the tri-nucleate pollen to become less viable, whereas a surplus of respiratory substrates causes the binucleate pollen to be more viable.

Even though colder temperatures typically help pollen store better, they can also cause pollen damage. However, the presence of these endogenous respiratory substrates in substantial amounts in the pollen of grass species, particularly starch, may suggest a failure in their utilisation rather than a

lack, suggesting that some degrading enzymes may have been rendered inactive. In fact, enzymes like amylases and phosphatases showed this type of decreased activity as early as 1951 [94]. Additionally, King [47] has demonstrated that the peroxidase reaction rate is probably a reliable predictor of pollen viability. The direct impact of these factors on pollen sterility has been confirmed by more current molecular studies that involve studying the structural rearrangements of mitochondrial genome and the activity of specific mitochondrial enzymes. To get the best results in these experiments, efforts must be made in the future to link these elements to pollen viability as well as storage conditions.

5. Conclusions

All of the described techniques show that pollen is used in gene-modification studies. Future research may concentrate on better comprehending the mechanisms causing damage to pollen membranes, preserving the structural integrity of pollen as it relates to its longevity, and improving storage techniques to make the most of the various pollen preservation techniques currently in use.

References

[1] K.R. Shivana, H.Y.M. Ram, Pollen biology: Contribution to fundamental and applied aspects, *Current Sci*. **65** (1993) 226-233.

[2] M. Kroh, Fine structure of Petunia pollen germinated in vivo, *Rev. Paleobot Palynol*. **3** (1967) 197-203.

[3] M.L. Wang, E.M. Hsu, L.E. Chang, C.S. Wang, T.H. Su, Y.J. Huang, L. Jiang, G.Y. Jauh, Gene expression profiles of cold-stored and fresh 'Pollen to investigate pollen germination and growth, *Plant Cell Phystol*. **45** (2004) 1519-1528.

[4] D.B. Dickinson, Permeability and respiratory properties of germinating pollen, *Physiol Plant*. **20** (1967) 118-27.

[5] C.M. Rounds, L.J. Winship, P.K. Hepler, Pollen tube energetic: respiration, fermentation and race to the ovule, *AOB Plants* (2011) pp.1-14.

[6] L.C.T. Yaung, R.G. Stanley, Incorporation of tritiated nucleosides thymidine, uridine and cytidine in nuclei of germinating pine pollen, *Nucleus* **6** (1963) 83-90.

[7] L.F. Molly, A.T. Wilson, Studies on initial biochemical reaction in the germination of pollen grains, *J. Exp. Bot*. **20** (1969) 457-465.

[8] T. Ischebeck, L. Valledor, D. Lyon, S. Gingl, M. Nayler, M. Meijon, V. Egelhofer, W. Weckwerth, Comprehensive cell specific protein analysis in early and late pollen development from diploid microsporocytes to pollen tube growth, *Mol. Cell Proteomics* **13** (2014) 295-310.

[9] R.K. Kamboj, H.F. Linskens, J.F. Jackson, Energy-driven protein release from germinating pollen of Petunia hybrid, *Ann. Bot*. **54** (1984) 647-52.

[10] J.R. Anderson, W.S. Barens, S. Bedinger, 2, 6-dichlorobenzonitrile, a cellulose biosynthesis inhibitor, affects morphology and structural integrity of petunia and lily pollen tubes, *J. Plant Physiol*. **159** (2002) 61-67.

[11] H.F. Linskens, J.A.M. Schrauwen, The release of free amino acids from germinating pollen, *Acta Bot. Neerl*. **18** (1969) 605-614.

[12] P.H. Brown, L. Ferguson, G. Picchioni, Boron nutrition of pistachio: Third year report, California Pistachio Industry, Annual Report- Crop Year 1992-1993 (1994) pp. 60-63.

[13] Q. Wang, L. Lu, Y. Li, J. Lin, Boron influences pollen germination and tube growth in Picca meyeri, *Tree Physiology* **23** (2003) 345-51.

[14] I. Acar, Effects of pistachio (Pistacia vera L.) pollinator types selected in Ceylanpinar on fruit set and fruit quality of some pistachio cultivars, PhD thesis, University of Cukurova, Adana, Turkey (2004).

[15] T.L. Holdaway-Clarke, P.K. Hepler, Control of pollen tube growth: role of ion gradients and fluxes, *New Phytol*. **159** (2003) 539-563.

[16] A.J. Richards, Plant Breeding Systems, George Allen Unwin, London, England (1986).

[17] C.P. Malik, A.K. Alhuwalia, Male sterility, in Recent Advances in Pollen Research, T.M. Varghese (Ed.), Allied Publishers, New Delhi (1985) pp. 171-200.

[18] B.M. Johri, I.K. Vasil, Physiology of pollen, *Bot. Rev*. **27** (1961) 325-381.

[19] D.P. Singh, A.M. Jermakow, S.M. Swain, Gibberellins are required for seed development and pollen tube growth in Arabidopsis, *Plant Cell* **14** (2002) 3133-3147.

[20] I.S. Bhandal, C.P. Malik, Effect of gibberellic acid, (2-chloroethyl) phosphoric acid, actinomycin-D and cycloheximide on the activity and leaching of some hydrolases in pollen suspension cultures of Crotalaria juncea, *Physiol. Plant* **45** (1979) 297-300.

[21] H. Hao, Y. Li, Y. Hu, J. Lin, Inhibition of RNA and protein synthesis in pollen tube development of pinus by actinomycin D and cycloheximide, *New Phytologist* **165** (2005) 721-730.

[22] J.P. Mascarenhas, Molecular mechanisms of pollen tube growth and differentiation, *Plant Cell* **5** (1993) 303-314.

[23] D.D. Fernando, J.N. Owens, X.S. Yu, A.K.M. Ekramoddoullah, RNA and protein synthesis during in vitro pollen germination and tube elongation in Pinus monticola and other conifers, *Sex Plant Reprod*. **13** (2001) 259 –264.

[24] J.J. Song, K. Nada, S. Tachibana, Suppression of S-adenosylmethionine decarboxylase activity is a major cause for high-temperature inhibition of pollen germination and tube growth in tomato (Lycopersicon esculentum Mill.), *Plant Cell Physiology* **43** (2002) 619 – 627.

[25] I.N. Roberts, G. Harrod, H.G. Dickinson, Pollen–stigma interactions in Brassica oleracea. II. The fate of stigma surface proteins following pollination and their role in the self-incompatibility response, *J. Cell Sci*. **66** (1984) 255 –264.

[26] A. Speranza, V. Scoccianti, R. Crinelli, G.L. Calzoni, M. Magnani, Inhibition of proteasome activity strongly affects kiwifruit pollen germination. Involvement of the ubiquitin/proteasome pathway as a major regulator, *Plant Physiology* **126** (2001) 1150 –1161.

[27] Y. Iwanami, Viability of pollen grains in organic solvents, *Botanique* **3** (1972) 61-68.

[28] Y. Iwanami, Retaining the viability of Camellia japonica pollen in various organic solvenis, *Plant Cell Physiol*. **13** (1972) 1139-1141.

[29] Y. Iwanami, N. Nakamura, Storage in an organic solvent as a means for preserving viability of pollen grains, *Stain Technol*. **47** (1972) 137-139.

[30] R. Mishra, K.R. Shivanna, Efficacy of organic solvents for storing pollen grains of some leguminous taxa, *Euphytica* **31** (1982) 991-995.

[31] A. Jain, K.R. Shivanna, Storage of pollen grains in organic solvents: effects of organic solvents on leaching of phospholipids and its relationship to pollen viability, *Ann. Bot*. **61** (1988) 325-330.

[32] A. Jain, K.R. Shivanna, Storage of pollen grains in organic solvents: effects of, solvents on pollen viability and membrane integrity, *J. Plant Physiol*. **12** (1988) 499-502.

[33] M. Grewal, Physiological and biochemical changes accompanying pollen storage in some cereals, pulses and

vegetables, PhD Dissertation, Department of Botany, Punjab Agricultural University, Ludhiana (1988).
[34] B. Barnabas, Possibilities for storage of graminaceous pollen, Ph.D. Thesis, Hungarian Academy of Sciences Budapest (in Hungarian) (1982), pp. 1-156.
[35] M.D. Jones, L.D. Newell, Longevity of pollen and stigma of grasses, *Amer. Soc. Agron*. **40** (1948) 195-204.
[36] P.L. Pfahler, H.F. Linskens, In vitro germination and pollen tube growth of maize (Zea mays L.) Pollen. VIII. Storage temperature and pollen source effects. *Planta* **111** (1973) 253-259.
[37] S.C. Pokhriyal, K.S. Mangth, Effects of pollen storage on seed set in pearl millet, *Seed Res*. **7** (1979) 131-135.
[38] J. Buintink, M.M. Claessens, M.A. Hemminga, F.A. Hoekstra, Influence of water content and temperature on molecular mobility and intracellular grasses in seeds and pollen, *Plant Physiol*. **118** (1998) 531-541.
[39] H.Y. Cheng, Relation between storage stability and molecular mobility in cells of seed and pollen. **31** (2005) 221-226.
[40] E. Pacini, M. Guarnieri, M. Nepi, Pollen carbohydrates and water content during development, presentation and dispersal: a short review, *Protoplasma* **228** (2006) 73-77.
[41] L.E. Towill, Low temperature and freeze-(vacuum) drying preservation of pollen in cryopreservation of Plant Cells and Organs, K.K. Kartha (Ed.), CRC Press, FL (1985) pp. 171-198.
[42] R.K. Tyagi, T. Hymowitz, Pollen from Glycine species survive cryogenic exposure, *Cryoletters* **24** (2003) 119-124.
[43] Z. Wang, Y. Zou, X. Li, Q. Zhang, L. Chen, H. Wu, D. Su, Y. Chen, J. Guo, D. Luo, Y. Long, Y. Zhong, Y.G. Liu, Cytoplasmic male sterility of rice with boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes - via distinct modes of mRNA silencing, *Plant cell* **18** (2006) 676-687.
[44] R.J.C. Harris, Biological applications of freezing and drying, Academic Press, New York (1954).
[45] E.L. Sharp, F.G. Smith, Further study of the preservation of Puccinia uredospores, *Phytopatholog* **47** (1957) 423-429.
[46] A.M. Cook, T.M. Roberts, J.P. Widdowson, Freeze-drying and radiation protection, *Nature* **198** (1963) 194-195.
[47] J.R. King, Peroxidase reaction as an indicator of pollen viability, *Stain Technol*. **35** (1960) 225-227.
[48] E.H. Knowlton, Studies in pollen with special reference to longevity, *Cornell Univ. Agr. Exp.Sta. Memoirsr* **52** (1922) 747-794.
[49] A.J. Snope, J.H. Ellison, Storage of asparagus pollen under various conditions of temperature, humidity and pressure, *Proc. Am. Soc. Hort. Sci*. **83** (1963) 447-452.
[50] C.H. Hanson, Longevity of pollen and ovaries of alfalfa, *Crop Sci*. **1** (1961) 114-116.
[51] R.E.C. Layne, D.J. Hagedorn, Effect of vacuum-drying, freeze-drying, and storage environment on the viability of pea pollen, *Crop Sci*. **3** (1963) 433-436.
[52] G.K. Livingston, T.M. Ching, K.K. Ching, Vitality of freeze-dried Douglas-fir pollen, *Plant Physiol*. **37** (1962) 11-29.
[53] T.M. Ching, K.K. Ching, Freeze-drying pine pollen, *Plant Physiol*. **39** (1964) 705-709.
[54] J. Nath, J.A. Anderson, Effect of freezing and freeze drying on viability and storage of Liliurn longifolium and zea mays L. pollen, *Cryobiology*. **12** (1975) 81-88.
[55] B. Barnabas, G. Kovacs, Storage of pollen, in Pollen Biotechnology for Crop Production and Improvement, K.R. Shivanna, V.K. Sawhney (Eds.) Cambridge University Press, UK (1997) pp. 293-314.
[56] T.V. Filipova, Possibility for long-term storage of maize pollen at -196° C temperature (in Russian), *Nauka Novosibirsk* (1985) pp. 64-68.
[57] S. Ganeshan, Cryogenic preservation of papaya pollen, *Sci. Hort*. **28** (1986) 65-70.
[58] S. Ganeshan, M.P. Alexander, Fertilizing ability of cryopreserved grape (Vitis vinifera L.) pollen, Genome 30, Suppl.1 (1988) pp.464.
[59] S. Ichikawa, K. Kaji, Y. Kubota, Studies on the scorage of larch (Larix leptolepis) Pollen at superlow temperatures, *Bull. Hokkaido For. Exp. Stn*. **8** (1970) 11.
[60] K.R. Shivanna, B.M. Johri, The Angiosperm Pollen structure and Function, Wiley Eastern Ltd, New Delhi (1985).
[61] T.M. Ching, W.H. Slabaugh, X-ray diffraction analysis of ice crystals in coniferous pollen, *Cryobiology* **2** (1966) 321.
[62] C. Kerhoas, G. Gay, C. Dumas, Multidisciplinary approach to the study of the plasma membrane of Zea mays pollen during controlled dehydration, *Planta* **171** (1987) 1-10.
[63] K.R. Shivanna, N.S. Rangaswamy, Pollen Biology: A Laboratory Mannual, Springer-Verlag, Berlin, Heidelberg (1992).
[64] H.L.M.V. Vithanage, D.M. Alexander, Synchronous flowering and pollen storage techniques as aids to artificial hybridization in pistachio (Pistaciassp), *J. Hort. Sci*.**60** (1985) 107-113.
[65] W.M. King, Report of chief on seed divisions, in Report of the Commissioner of Agriculture (Yearbook), GPO, Washington, DC (1885) pp. 47-61.
[66] F.A. Hoekstra, J. Bruinsma, Control of respiration of binucleate and trinucleate pollen under humid conditions, *Plant Physiol*. **48** (1980) 71-77.
[67] M. Gonzalez-Sanchez, M. Rosato, M. Chiavarino, M.J. Puertas, Chromosome instabilities and programmed cell death in tapetal cells of maize with B chromosomes and effects on pollen viability, *Genetics* **166** (2004) 999-1009.
[68] J.H. Crowe, F.A. Hoekstra, L.M. Crowe, Membrane phase transitions are responsible for imbibitional damage in dry pollen, *Proc. Nat. Acad. Sci*. **86** (1989) 520523.
[69] J.H. Crowe, F.A. Hoekstra, L.M. Crowe, T.J. Achordoguy, E. Drobnis, Lipid phase transitions measured in intact cells with cells with Fourier transform infrared spectroscopy, *Cryobiology* **26** (1989) 76-83.
[70] M. Nepi, G.G. Franchi, E. Pacini, Pollen hydration status at dispersal: cytophysiological features and strategies, *Protoplasma* **216** (2001) 171-180.
[71] G.G. Franchi, M. Nepi, A. Dafni, E. Pacini, Partially hydrated pollen: taxonomic distribution, ecological and evolutionary significance, *Plant System. Evol*. **234** (2002) 211-227.
[72] Y. Heslop-Harrison, Control gates and micro-ecology: The pollen-stigma interaction in perspective, *Ann. Bot.* **85** (2000) 5-13.
[73] V.S. Luna, M.J. Figueroa, M.B. Baltazar, L.R. Gomez, R. Townsend, J.B. Schoper, Maize pollen longevity and distance isolation requirements for effective pollen control, *Crop Sci*. **41** (2001) 1551-1557.
[74] D.E. Aylor, Survival of maize (Zea mays) pollen exposed in the atmosphere, *Agri. Forest Meteor*. **123** (2004) 125-133.
[75] D.E. Aylor, Rate of dehydration of corn (Zea mays L.) pollen in the air, *J. Exp. Bot*. **54** (2003) 2307-2312.
[76] E. Pacini, Types and meaning of pollen carbohydrate reserves, *Sex. Plant Reprod*. **9** (1996) 362-366.
[77] F.A. Hoekstra, D. Van, E.W. Wal, Initial moisture content and temperature of imbibition determine extent of imbibitional injury in pollen, *Plant Physiol*. **133** (1989) 257-262.
[78] J.J. Burke, Moisture sensitivity of cotton pollen: an emasculation tool for hybrid production, *Agron J*. **94** (2002) 883-888.
[79] M. Pfundt, Der Einfluss der Luftfeuchtigkeit auf die Lebensdauer des Blutenstaubes, *Jahrb. Wiss. Bot*. **47** (1910) 1-40.
[80] A. Manaresi, Richerche sulla longevita del polline di alcune plante de frutto, *Stat. Sper.Agr. Ital*. **48** (1924) 33-35.

[81] J.R. King, The freeze-drying of pollens, *Econ. Botany* **15** (1961) 91-98.

[82] N.E. Pfeiffer, longevity of pollen of Lilium and hybrid Amaryllis, *Contribn. Boyce Thompson Inst*. **9** (1936) 199-211.

[83] S. Sato, M.M. Peet, J.F. Thomas, Determining critical pre- and post-anthesis periods and physiological processes in Lycopersicon esculentum Mill. exposed to moderately elevated temperatures, *J. Exp. Bot*. **53** (2002) 1187-1195.

[84] G.U. Rao, A. Jain, K.R. Shivanna, Effects of high temperature stress on Brassica pollen: viability, germination and ability to set fruits and seeds, *Ann. Bot*. **69** (1992) 193-198.

[85] T.G. Porch, M. Jahn, Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of Phaseolus vulgaris, *Plant Cell Environ*. **24** (2001) 723-731.

[86] M.M. Caldwell, L.O. Bjorn, J.F. Bornman, S.D. Flint, G. Kulandaivelu, A.H. Teramura, M. Tevini, Effects of increased solar ultraviolet radiation on terrestrial ecosystems, *J. Photochem. Photobio*. **46** (1998) 40-52.

[87] T.M. Murphy, Membranes as targets of ultraviolet radiation, *Physiologia Plantarum* **58** (1983) 381-388.

[88] A. Dafni, D. Firmage, Pollen viability and longevity: Practical, ecological and evolutionary implications, *Plant Syst. Evol*. **222** (2000) 113-132.

[89] N. Nielsen, Vitamin content of pollen after storing, *Acta Chem. Scand*. **10** (1956) 332-333.

[90] J. Buitink, C. Walters-Vertucci, F.A. Hoekstra, O. Leprince, Calorimetric properties of dehydrating pollen (Analysis of a desiccation to lerant and an intolerant species), *Plant Physiol*. **111** (1996) 235-242.

[91] A.T. Wilson, M. Vickers, L.R.B. Mann, Metabolism in dry pollen: a novel technique for studying anhydrobiosis, *Naturwissenschaften* **66** (1979) 53-54.

[92] R.G. Stanley, H.F. Linskens, Pollen: Biology, Biochemistry and Management, Springer-Verlag, Berlin (1974).

[93] E.G. Wang, W.Q. Song, Identification of PCR markers associated with cytoplasmic male sterility in Brassica oleraceae var botrytis, *Chaun*. **27** (2005) 236-40.

[94] A. Hoeckel, Beitrag zen Kenntnis pollen-fermente, *Planta* **39** (1951) 431-459.

Publisher's Note: Research Plateau Publishers stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.