Pak. J. Bot., 46(3): 951-956, 2014.

IN VITRO POLLEN GERMINATION OF FIVE *CITRUS* SPECIES

SHAUKAT ALI KHAN AND ANJUM PERVEEN

Centre for Plant Conservation, University of Karachi
Department of Botany, University of Karachi, Karachi 75270, Pakistan.

Abstract

The aim of present study is *In vitro* germination of the pollen grains of five *Citrus* species belonging to the family Rutaceae *viz*., *Citrus aurantium* L. var., *aurantium* Hook.f., *C. limon* (L.) Brum. f., *C. paradisii* Macfad, *C. reticulata* Blanco and *C. sinensis* (L.) Osbeck. using "hanging drop" technique. The germination was checked up to 48 weeks, for the pollen stored at different temperatures like 4ºC, -20ºC, -30ºC and -60ºC. The study indicates that low temperature and low relative humidity is better than high temperature and humidity with respect to pollen germination capacity and viability. Freeze dryer (-60ºC) seems to be the best method to maintain pollen viability of stored pollen grains for a long period of time. Among five species *Citrus aurantium*, *C. limon* and *C. sinensis* showed high percentage of germination as compared to *C. reticulata* and *C. paradisii.*

Introduction

Rutaceae is a large, predominantly tropical and subtropical family, consisting of 150–162 genera and 1500–2096 species, with three main centers of diversity Tropical America, southern Africa, and Australia (Kubitzki *et al*., 2011). The family is economically important for edible fruits (especially *Citrus*, with many varieties of oranges, lemons, tangerines, etc.), aromatic oils (*Boronia* and *Ruta*), drugs (*Pilocarpus*, source of pilocarpine, used against glaucoma), and bitter beverages used to treat fevers (*Angostura*, *Galipea*). Species of *Flindersia*, *Zanthoxylum*, *Balfourodendron*, and *Euxylophora* are sources of timbers. More recently, the antimicrobial and antifungal properties of rutaceous compounds are being exploited as natural pesticides (Oliva *et al*., 2000), herbicides and antimicrobials (Mandalari *et al*., 2007), while others are medicinally useful (Moraes *et al*., 2003).

In Pakistan Rutaceae is represented by 11 genera and 27 species while the genus *Citrus* is represented by 10 species. Most or all the species of the genus *Citrus* are neutralized cultivated and derived from the native species of tropical and sub-tropical regions of SE. Asia because of great economic importance, domestication, cultivation and hybridization led to many varieties (Hassan & Ghazanfar, 1980).

Citrus fruits have been recognized as an important source of taste, food and integrated as a part of our daily diet, playing key role in providing energy, nutrients and in health promotion. *Citrus* fruits have low proteins and little fat content, they mainly supply carbohydrates (Sucrose, Glucose, Fructose). Fresh *Citrus* fruits are also a good source of dietary fibers, which is associated with gastrointestinal diseases, and also lower the circulating cholesterol. Along with abundant vitamin "C" the fruits also contain Vitamins "B" (Thiamine, Pyrimidine, Niacin, Riboflavin, Pantothenic acid and Folate). Phytochemicals like carotenoids, flavonoids and limonoids are of vital importance in promoting human health due to their antioxidant properties and protection from various chronic diseases. Vitamin "C" prevents blood shortage and makes the bones, teeth stronger and healthier. *Citrus* juices not only promote skin but also increase hunger (Anon., 1994).

To increase the yield and requirement of desired pollen, genetic conservation and storage is desirable for plants breeders, since pollen is known to transmit important heritable characters. Plant breeders could have access to a facility called pollen bank, from where any body can draw pollen of its own choice in the process of breeding a new cultivar. Several methods of pollen storage have been tried and employed of which the most important factors are controlled temperature and humidity. The literature on pollen storage has been reviewed by Visser, (1955), Stanley & Linskens, (1974), Goff, (2001), Aslantus & Pirlak, (2002), Zhang, (2002), Bomben *et al*., (2006), Song & Tachibana, (2007) and Dutta *et al*., (2013). The storage life of Clementine Manderinin (*C. reticulata*) and *Poncirus trifoliate* pollen was effectively extended in oxygen free atmosphere (Sahar *et al*., 1980). Different investigators have same consensus that low temperature and humidity are the two major influencing factors in storage of pollen grains for a long period of time (King, 1961; Ganeshan, 1986; Gill *et al*., 1992; Malik & Thind, 1992; Shivanna & Rangaswamy, 1992; Mesejo *et al*., 2006; Janick *et al*., 2010). Pollen grains can germinate and grow readily in water or in sugar medium, their growth can be accelerated by adding vitamins and microelements such as boron, magnesium, calcium, potassium etc. Pollen physiology especially germination and viability has received considerable attention for its application in plant breeding, conservation, adaptation and understanding of the physiological behaviour of the fertilizing pollen grains.

There are several reports on pollen germination and viability of different taxa (Kapoor, 1976; Cohen *et al*., 1988; Eti & Stosser, 1992; Mercado *et al*., 1994; Sato *et al*., 1998; Dafni & Firmage, 2000; Vaknin *et al*., 2003; Ateyyeh, 2005; Bermejo *et al*., 2011 and Khan *et al*., 2013) with varied aims and objectives, however there is little information about the pollen germination of temperate vegetables, fruits and ornamental plants. Nair (1964) correlated pollen morphology of *Vitis vinifera* with physiological potential by studying pollen germination in different types. Mesejo *et al*., (2006) found out the inhibitory effect of CuSo4 on *In vitro* pollen germination of "Manderin" and after 8 hrs of incubation the development of pollen tube was stopped.

The *In vitro* pollen germination of *Citrus maxima* and *C. paradisi* was suppressed by adding olive oil to a medium containing 0.8% agar, 10% sucrose and 50ppm Citric acid, but the germination was significantly increased in *C. maxima* in a medium having 0.8% agar and 20% sucrose (Ateyyah, 2005).

This is the first attempt to study the *In vitro* pollen germination of five *Citrus* species from Pakistan. Pollen germination of these five *Citrus* species was assessed to develop a pollen bank from where desired pollen may be provided to farmer for hybridization.

Materials and Methods

In flowering period of the five *Citrus* species pollen were collected in large quantity from cultivated fields of Kalat (Khan of Kalat fields), Sindh (Khirpur) and KPK (Swat, Buner). Pollen grains were stored in different storage conditions in refrigerator, freezer and freeze drier but before that fresh pollen were systematically subjected to preliminary viability tests Alexander (1969). For Pollen germination media was prepared using standard technique of Brewbaker & Kwack (1963). The germination was scored after 6-8 hours of incubation at room temperature in humid chambers. Pollen tube equal to twice the diameter of pollen were counted as germinated while burst pollen were considered ungerminated. The viability of stored pollen was assessed in terms of percent germination. The pollen grains slides were also prepared for light microscopy (LM) using the standard procedure of Erdtman (1952). Observations were made with a Nikon type- 2 microscope.

Result and Discussion

***Citrus limon* (L.) Burm.f.:** *C. lemon* species showed 67.30% of germination in fresh pollen and after 4 weeks of storage nearly 70% germination was noted in all stored conditions, refrigerated (4ºC) freezed (-20ºC, -30ºC) and freeze dried (-60ºC) in 10% solution (Fig. 1). The refrigerated pollen showed 64.20% after 4 weeks and 26.50% after 48 weeks of storage in 10% and 30% solutions respectively (Table 1). Freezed pollen (-20ºC) showed 69.30% germination after 4 weeks in 10% solution as compared to 72.10% of the other freezed condition (-30ºC), while after 48 weeks the germination was 53.50% and 56.70% respectively. Freeze dried pollen (-60ºC) showed somewhat low germination after 4 weeks (67.30%) but after 48 weeks this condition showed more viability and germination, which was 62.00% in 20% solution, so it seemed to be a better method as compared to refrigerated and freezed. 10% solution may be favourable for pollen germination. As the time progressed pollen needed more concentrated solutions in late weeks contrast to early weeks (Table 1).

***C. aurantium* L. var., *aurantium* Hook.f.:** The bitter orange showed 60.30% germination which is more than that of lemon but lower germination and viability was observed in stored pollen (Fig. 2). After 4 weeks of storage the refrigerated pollen showed 58% of germination in 10% solution and after 48 weeks the germination was 27.00% in 40% solution. The freeze conditions (-20ºC, -30ºC) showed ›60% germination after 4 weeks of storage in 10% solutions and after 48 weeks percentage of germination was 29.40% and 30.00% in 20% solution. Freeze dried pollen (-60ºC) showed 69.10% of germination in 10% solution which is the highest after 4 weeks, while after 48 weeks 63.00% germination was noted in 20% solution. So it seemed that 10% solution is good in the start but later on 20% solution showed better results and freeze dried (-60ºC) looks to be better condition as it could maintain the viability for a longer period (Table 2).

***C. paradisii* Macfad:** The grape fruit showed 50.60% of germination in fresh pollen and after 4 weeks of storage above 60% germination was noted in both refrigerated (4ºC) and freezed pollen (-20ºC, -30ºC) in 10% solution (Fig. 3). Unexpectedly freeze dried pollen (-60ºC) showed 49.00 % of germination in 10% solution. Refrigerated pollen showed 59.00% of germination in 10% solution and after 48 weeks only 3.10% of germination was observed. The pollen in -20ºC and -30ºC showed 44.20% and 48.00% of germination in 10% solution after 4 weeks respectively, while 11.20% and 16.00% germination was observed after 48 weeks of storage (Table 3). Again freeze dried pollen showed good potential to maintain pollen viability, after 4 weeks of storage 50.10% of germination was noted and after 48 weeks the germination was 42.70% (Table 3). Grape fruit showed somewhat poor pollen germination compared to other *Citrus* species.

***C. reticulata* Blanco:** Tangerine also showed better pollen germination like lemon. The fresh pollen showed 50.00% of germination, while both refrigerated (4ºC) and freezed pollen (-20ºC, -30ºC) showed more than 60% of germination after 4 weeks of storage in 10% solution (Fig. 4). Like grape fruit the freeze dried pollen of tangerine also showed 49.00% germination after 4 weeks as compared 63.40% and 63.00% in freezed pollen(-20ºC, -30ºC) in 10% respectively. As viability concerned freeze dried pollen showed better germination 39.00% as compared to 24.70%, 34.50% and 22.69% in refrigerated and freezed pollen (-20ºC, -30ºC). Like other species with the passage of time more concentrated solutions showed better germination like 30%, and 40% (Table 4).

***C. sinensis* (L.) Osbeck:** Pollen of Sweet orange showed more or less similar results like lemon both in fresh and stored conditions in early weeks of storage. The fresh pollen showed 62.00% of germination and after 4 weeks the refrigerated pollen showed 61.50% germination in 10% solution and 23.40% after 48 weeks (Fig. 5). Freezed pollen (-20ºC) showed 65.20% of germination after 4 weeks in 10% solution and 26.00% after 48 weeks of storage, the other freeze condition (-30ºC) showed poor results after 4 weeks 45.60% of germination was noted and 7.20% after 48 weeks in 10% solution. Freeze dried (-60ºC) showed highest 72.00% of germination after 4 weeks and 20.00% after 48 weeks of storage in 10% and 30% solutions respectively (Table 5).

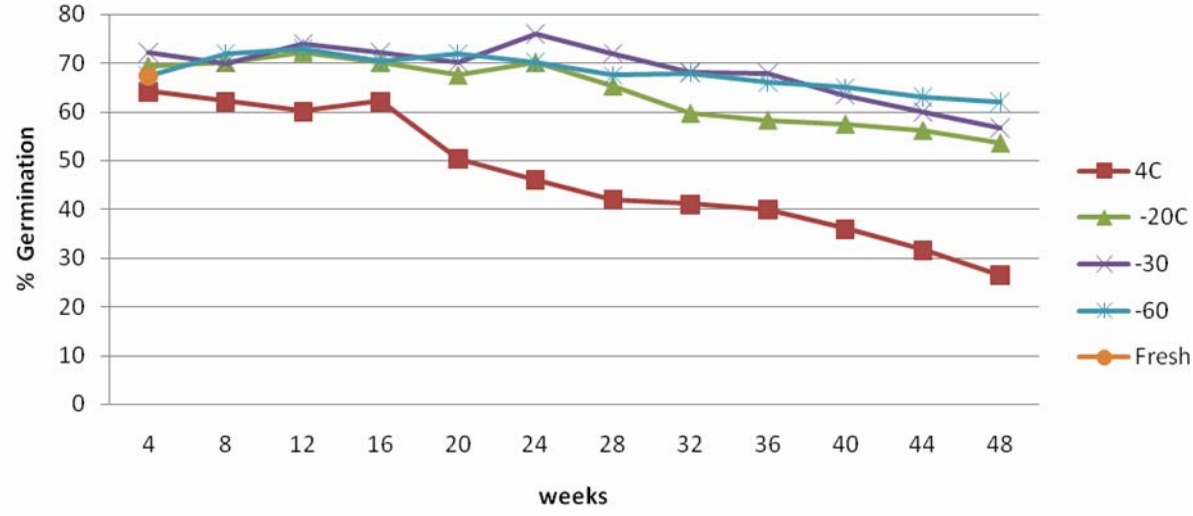

Fig. 1. Germination percentage of *Citrus limon* (L.) Brum.f. (Rutaceae) pollen at different weeks.

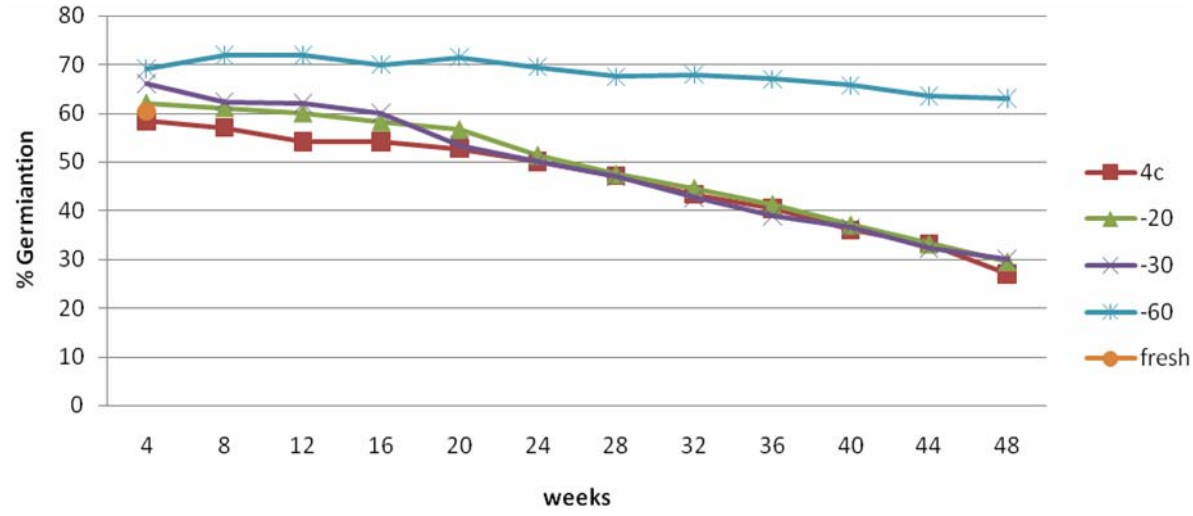

Fig. 2. Germination percentage of *C. aurantium* L. (Rutaceae) pollen in different weeks.

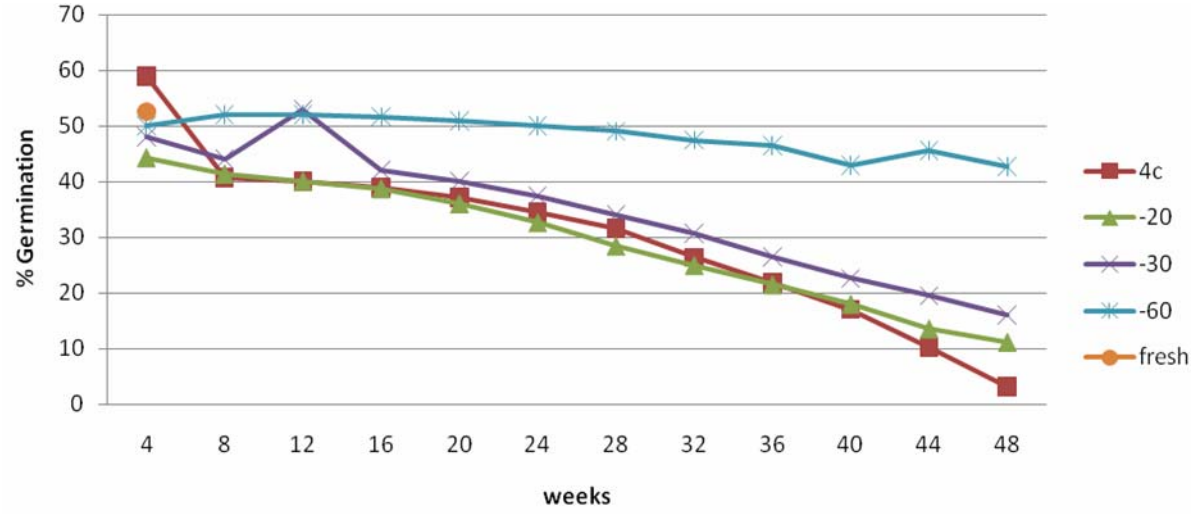

Fig. 3. Germination percentage of *C. paradisii* Macfad. (Rutaceae) pollen in different weeks.

Table 1. Germination capacity of *Citrus limon* (L.) Brum.f. (Rutaceae) pollen in different sucrose and boric acid solutions.

Weeks	Germination % at storage temperature 4ºC	Germination noted in % solution	Germination % at storage temperature -20ºC	Germination noted in % solution	Germination % at storage temperature -30ºC	Germination noted in % solution	Germination % at storage temperature -60ºC	Germination noted in % solution
4	64.20	10	69.30	10	72.10	10	67.30	10
8	62.10	10	70.00	20	70.00	10	72.00	10
12	60.10	10	72.10	10	74.00	10	73.00	10
16	62.10	10	70.00	10	72.10	10	70.40	10
20	50.40	20	67.40	10	70.10	20	72.00	10
24	46.10	20	70.00	10	76.00	10	70.10	10
28	42.00	20	65.20	10	72.00	10	67.50	10
32	41.10	20	59.60	10	68.20	10	68.00	20
36	40.00	30	58.20	40	68.00	10	66.00	10
40	36.00	30	57.40	20	63.40	10	65.00	30
44	31.70	30	56.10	20	60.00	10	63.10	10
48	26.50	30	53.50	30	56.70	30	62.00	20

Percentage of germination at fresh: 67.30%

Table 2. Germination capacity of *C. aurantium* L. (Rutaceae) pollen in different sucrose and boric acid solutions.

Weeks	Germination % at storage temperature 4ºC	Germination noted in % solution	Germination % at storage temperature -20ºC	Germination noted in % solution	Germination % at storage temperature -30ºC	Germination noted in % solution	Germination % at storage temperature -60ºC	Germination noted in % solution
4	58.40	10	62.00	10	66.10	10	69.10	10
8	57.00	20	61.10	10	62.40	10	72.00	10
12	54.10	10	60.00	10	62.00	10	72.00	10
16	54.00	20	58.20	10	60.00	10	70.00	10
20	52.70	20	56.60	30	53.40	10	71.60	10
24	50.00	20	51.30	20	50.10	30	69.50	10
28	47.10	30	47.40	20	47.10	20	67.50	20
32	43.30	30	44.50	20	42.60	20	68.00	20
36	40.50	30	41.20	20	39.00	20	67.00	20
40	36.00	30	37.00	20	36.50	20	65.70	20
44	33,20	30	33.10	20	32.40	20	63.60	20
48	27.00	40	29.40	20	30.00	20	63.00	20

Percentage of germination at fresh: 60.30%

Table 3. Germination capacity of *C. paradisii* Macfad. (Rutaceae) pollen in different sucrose and boric acid solutions.

Weeks	Germination % at storage temperature 4ºC	Germination noted in % solution	Germination % at storage temperature -20ºC	Germination noted in % solution	Germination % at storage temperature -30ºC	Germination noted in % solution	Germination % at storage temperature -60ºC	Germination noted in % solution
4	59.00	10	44.20	10	48.00	10	50.10	10
8	40.70	10	41.30	10	44.10	10	52.00	10
12	40.00	20	40.00	10	53.00	10	52.10	10
16	39.00	20	38.70	10	42.10	10	51.70	10
20	37.20	20	36.00	10	40.00	10	51.00	10
24	34.50	20	32.60	20	37.50	10	50.00	10
28	31.60	20	28.40	20	34.00	10	49.10	20
32	26.40	30	25.00	20	30.70	20	47.50	20
36	21.80	30	21.50	20	26.50	30	46.50	20
40	17.00	40	18.00	20	22.60	30	43.00	20
44	10.20	40	13.60	20	19.50	30	45.60	20
48	03.10	40	11.20	30	16.00	30	42.70	20

Percentage of germination at fresh: 52.60%

Table 4. Germination capacity of *C. reticulata* Blanco (Rutaceae) pollen in different sucrose and boric acid solutions.

Weeks	Germination % at storage temperature 4ºC	Germination noted in % solution	Germination % at storage temperature -20ºC	Germination noted in % solution	Germination % at storage temperature -30ºC	Germination noted in % solution	Germination % at storage temperature -60ºC	Germination noted in % solution
4	60.00	10	63.40	10	63.00	10	49.00	10
8	52.60	20	62.00	10	60.20	10	50.10	10
12	52.00	20	61.00	10	59.00	10	51.00	10
16	50.30	20	57.60	10	55.70	10	50.70	10
20	47.40	20	54.00	10	53.00	10	50.00	10
24	45.90	30	50.10	10	50.90	10	49.60	10
28	42.00	30	46.00	20	46.40	10	48.00	10
32	39.30	40	42.10	20	41.70	20	46.70	20
36	35.00	40	41.00	20	37.60	30	45.00	20
40	32.40	40	39.60	20	32.40	30	42.70	20
44	27.00	50	36.50	20	27.60	30	40.60	20
48	24.70	40	34.50	30	22.60	30	39.00	30

Percentage of germination at fresh: 50.00%

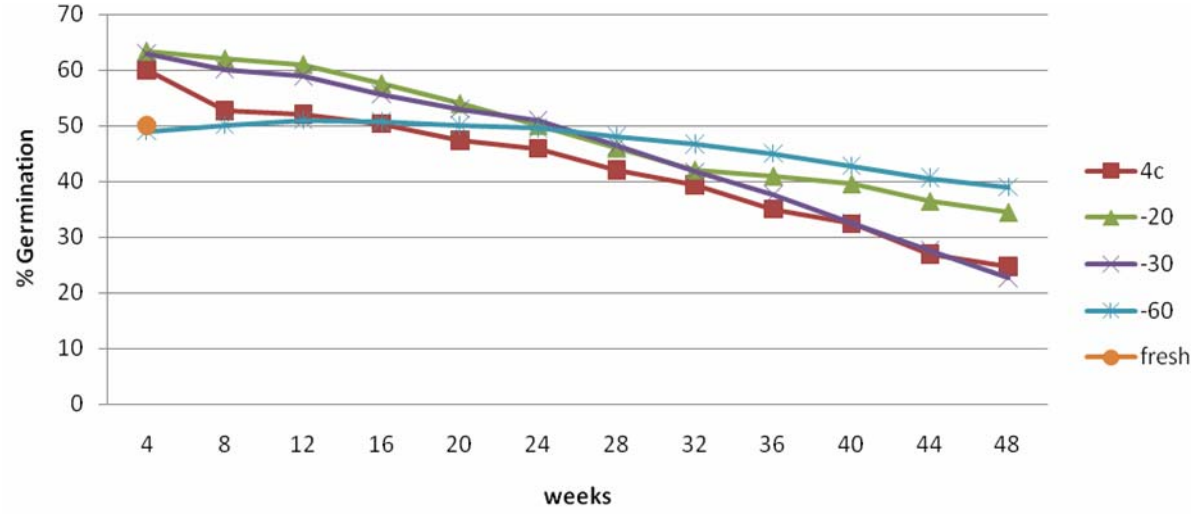

Fig. 4. Germination percentage of *C. reticulata* Blanco (Rutaceae) pollen in different weeks.

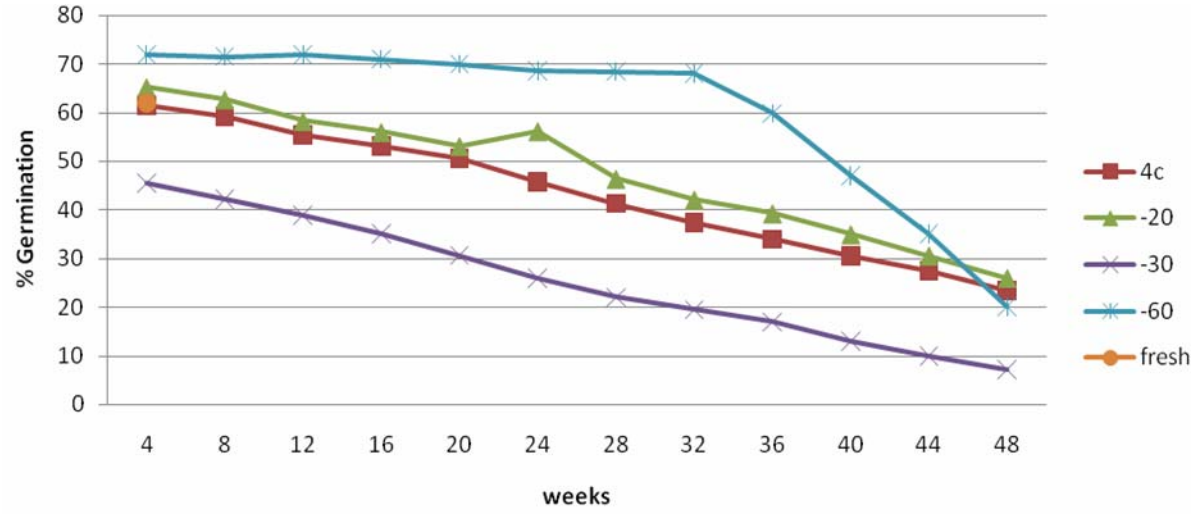

Fig. 5. Germination percentage of *Citrus sinensis* (L.) Osbeck. (Rutaceae) pollen in different weeks.

Table 5. Germination capacity of *Citrus sinensis* (L.) Osbeck. (Rutaceae) pollen in different sucrose and boric acid solutions.

Weeks	Germination % at storage temperature 4ºC	Germination noted in % solution	Germination % at storage temperature -20ºC	Germination noted in % solution	Germination % at storage temperature -30ºC	Germination noted in % solution	Germination % at storage temperature -60ºC	Germination noted in % solution
4	61.50	10	65.20	10	45.60	10	72.00	10
8	59.10	10	62.70	10	42.30	10	71.60	10
12	55.40	10	58.30	10	39.00	10	72.00	10
16	53.10	20	56.00	10	35.10	10	71.00	10
20	50.50	20	53.10	10	30.70	10	70.00	10
24	45.70	20	56.20	10	26.00	10	68.60	10
28	41.30	20	46.40	20	22.10	10	68.50	10
32	37.40	20	42.10	20	19.60	20	68.10	10
36	34.00	30	39.30	20	17.00	10	59.90	20
40	30.60	30	35.00	30	13.10	10	47.00	20
44	27.50	30	30.60	30	10.00	20	35.10	20
48	23.40	40	26.00	30	07.20	20	20.00	30

Percentage of germination at fresh: 62.00%

Conclusion

The study indicates that *Citrus limon* showed better percentage of germination as compared to other species. At fresh 67.30% germination was noted as compared to that of 60.30% of *C. aurantium*, 52.60% of *C. paradisii*, 50.00% of *C. reticulata* and 62.00% of *C. sinensis*. At stored conditions (4C, -20C, -30C and -60C) limon also showed better percentage of germination comparatively as well as the pollen showed viability for a longer period. Among five *Citrus* species *C. limon*, *C. sinensis* and *C.*

aurantium showed better percentage of germination and viability after 48 weeks. 10% and 20% solutions showed better results but occasionally 30% and 40% also showed reasonably good at results later weeks.

Acknowledgement

We are thankful to PSF (Pakistan Science foundation) for providing financial support for this project.

References

Alexander, M.P. 1969. Staining of aborted and non-aborted pollen. *Stain Technology*, 44: 117-122.

Anonymous. 1994. Nutritional and health benefits of *Citrus* fruits.

Aslantus, R. and L. Pirlak. 2002. Storage of Strawberry pollen. *Acta. Hort*., 567: IV *International Strawberry Symposium.*

Ateyyeh, A.F. 2005. Improving *In vitro* pollen germination of five species of fruit trees. *Agri. Sci*., 33(2): 189-194.

Bermejo, Almudena J. Pardo, Cano and Antonio. 2011. Infulence of gamma irradiation on seedless *Citrus* production: pollen germination and fruit quality. *Food and Nutrition Science*, 2(3): 169-180.

Bomben, C., C. Malossini, G. Cipriani and R. Testolin. 2006. Long term storage of Kiwifruit pollen. *Acta Hort*., 498: IV International Symposium on Kiwifruit.

Brewbacker, J.L. and B.H. Kwack. 1963. The essential role of calcium ion in pollen tube growth. *Amer. J. Bot.*, 50: 859-865.

Cohen, E., U. Lavi and P. Spiegel-Roy. 1988. Crygeneic preservation of papaya pollen. *Sc. Horti*., 28: 65-70.

Dafni A. and D. Firmage. 2000. Pollen viability and longevity: practical, ecological and evolutionary amplications. *Pl. Syst. Evol*., 222: 113-132.

Dutta, S.K., M. Srivatav, R. Chaudhary, K. Lal, P. Patil. S.K. Sing and A.K. Sing. 2013. Low temperature storage of mango (*Mangifera indica* L.), pollen. *Sci. Hort*., 161: 193-197.

Erdtman, G. 1952. *Pollen morphology and Plant Taxonomy*. Angiosperms. *Almqvist & Wiksell*, Uppsala.

Eti, S. and R. Stosser. 1992. Pollen tube growth and development of ovules in relation to fruit set in Mandarines, cv, Clementine (*Citrus reticulata* Blanco). *Acta Hort*. (ISHS), 321: 621-625.

Ganshan, S. 1986. Cryogenic preservation of papaya pollen. *Sci. Hort*., 28(1-2): pp. 65-70.

Gill, M.S. Neelam and C.P. Malik. 1992. Pollen biotechnology storage and viability. In: *Pollen Physiology and Biotechnology*. (Ed.): C.P. Malik. Today and tomorrow's Printer and Publisher, New Delhi, India.

Goff, E.S. 2001. A study of certain conditions affecting setting of fruits. *Wis. Agr. Exp. Sta*., *Rep*., 18: 289-30.

Hassan, U.D. and S.A. Ghazanfar (Rutaceae). 1980. *Flora of Pakistan* Univ. of Karachi, Karachi.

Janick, J.W.W. Hanna and L. E. Towill. 2010. Long term pollen storage. *Plant Breeding Review*, 13.

Kapoor, S.K. 1976. Pollen germination in some Cucurbits. *J. Palyn*., 12(1&2): 87-93.

Khan, S.A., A. Perveen and G.R. Sarwar. 2013. Germination capacity and viability in pollen of *Prunus amygdalus Batsch* (Rosaceae). *Pak. J. Bot.*, 45(4): 1383-1385.

King. J.R. 1961. The freeze drying of pollen. *Economic Botany*, 15: 91-93.

Kubitzki, K., J.A. Kallunki, M. Duretto and P.G. Wilson. 2011. Rutaceae. In: *The families and Genera of Vascular Plants*, (Ed.): K. Kubitzki. 10: Flowering Plants: Eudicots (Sapindales, Cucurbitales, Myrtaceae). Berlin and Heidelberg, Germany, *Springer Heidelberg*, pp. 276-356.

Malik, C.P. and S.K. Thind. 1992. Pollen biotechnology and fertilization engineering in crop improvement. In: *Pollen Physiology and Biotechnology*. (Ed.): C.P Malik. New Delhi, India.

Mandalari, G., R.N. Bennett, G. Bisignano, D. Trombetta, A. Saija, C.B. Faulds, M.J. Gasson and A. Narbad. 2007. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *Journal of Applied Microbiology*, 103: 2056-2064.

Mercado, A., R. Fernandaz, A. Miguel and Quesda.1994. *In vitro* growth of pepper pollen in liquid medium. *Scientia Hort*., 52: 273-281.

Mesejo, C., A.M. Fuentes, C. Reig and R. Fernando. 2006. The inhibitory effect of CuSo4 on *Citrus* pollen germination and pollen tube growth and its application for the production of seedless fruit.

Moraes, V.R.S., D.M. Tomaleza, R.J. Ferracin, C.F. Garcia, M. Sannomiya, M.P.C. Soriano, M.F.G.F. Silva, P.C. Vieira, J.B. Fernandes, E. Rodrigues-Filho, E.G. Magalhães, A.F. Magalhães, E.F. Pimenta, D.H.F. Souza and G. Oliva. 2003. Enzymatic inhibition studies of selected flavonoids and chemosystematic significance of polymethoxylated flavonoids and quinoline alkaloids in *Neoraputia*. *Journal of the Brazilian Chemical Society*, 14: 380-387.

Nair, P.K.K. 1964. Pollen grains of Western Himaleyan plants. Asia Publishing House Bombay.

Oliva, A., B. Di Blasio, G. Cafiero, G. Aliotta, R. Iacovino and V. De Feo. 2000. Allelochemicals from rue (*Ruta graveolens* L.) and olive (*Olea europaea* L.) oil mill waste waters as potential natural pesticides. *Current Topics in Phytochemistry*, 3: 167-177.

Sato, S., M.M. Peet and J.F. Thomas. 1998. Establishment of reliable methods of *In vitro* pollen germination and pollen tube preservation of *Brassica rapa* (Syn. *B. campestris*). *Euphytica*, 103: 29-33.

Shivanna, K.R. and N.S. Ranaswamy. 1992. Pollen biology. *A laboratory manual. New Springer, Verlag*. Berlin, Heidelberg, New York. Stain Technology, 44: 117-122.

Song, J and S. Tachibana. 2007. Loss of viability of tomato pollen during long term dry storage is associated with reduced capacity for translating polyamine biosynthetic enzyme genes after rehydration. *J. Exp. Bot*., 58(15-16): 4235-4244.

Stanley, R.G. and H.F. Linskens. 1974. *Pollen biology, biochemistry and management. Springer, Verlag*. Berlin, Heidelberg, New York.

Vaknin, Y., D. Mills and A. Benzioni. 2003. Pollen production and pollen viability in male jojoba plants. *Industrial Crops and Products*, 18(2): 117-123.

Visser, T. 1955. Germination and storage of pollen. *Meded. Landb*. Hoogesch (Wageningen), 55: 1-68.

Zhang, L.Y. 2002. Conervation of pollen at low temperature and in liquid Nitrogen. *Acta. Hort. Sinica*, 2000-02.

(Received for publication 16 January 2013)