Pak. J. Bot., 45(4): 1383-1385, 2013.

GERMINATION CAPACITY AND VIABILITY IN POLLEN OF *PRUNUS AMYGDALUS BATSCH* (ROSACEAE)

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Abstract

Present investigation of pollen germination and viability pertain to *Prunus amygdalus* belonging to family Rosaceae. The pollen germination was examined up to 48 weeks in different concentrations of sucrose and boric acid solutions using "hanging drop technique". Viability was determined by storing pollen in different conditions as refrigerator (4ºC), freezer (-20ºC, -30ºC), freeze drier (-60ºC). Pollen stored at low temperature showed better percentage of germination compared to pollen stored at 4ºC and fresh. Freeze dried pollen showed viability for a longer period.

Introduction

Rosaceae is one of the largest family of dicot, sub-cosmopolitan in distribution particularly diversified in northern hemisphere. It includes approximately 100 genera and 3100 species (Judd *et al*., 1999 Mabberely, 2008). It includes many species of economic importance particularly edible fruits called stone fruits. Species of *Prunus* includes peach, cherry, plum, apricot and almond. Almond is the most important nut throughout the world and almond oil is a rich source of vitamin E and D and also contains essential minerals like Ca and Mg. Almond oil enhance the taste of food and has many cosmetic benefits to improve complexion, reduce skin irritation, inflammation, delay ageing process, help to nourish hairs and make it long and strong (www.Indiaparenting.com/health/generalhealth).Almond when incorporated in diet reported to reduce colon cancer in rats (Davis & Iwahashi, 2001) and increase HDL cholesterol and reduce LDL cholesterol level in human (Hyson *et al*., 2002). The nutritional values of almond fruit is related to its kernel, shells and hulls which are used as live stock feed and burned as a fuel. The genus *Prunus* is reported to have interesting biological properties such as sedative, anti-inflammatory, anti-hyperlipidemic, anti-tumoural and anti-toxidant activities (Donovan *et al*., 1998, Wang *et al*., 1999 & Chen *et al*., 2005).

Prunus amygdalus (almond) originates in Asia and it is ancient central Asian crops cultivated in Middle East and introduced in Spain during the incursions of Arabs into Europe in 8th century. Almond has been transferred into a large scale industry in California that now produces over 70% of the world crops and ranked as 7th largest US food export (Jahanban *et al*., 2009).

Pollen storage is a useful technique to establish a pollen bank which can be used by plant breeders for fertilization and hybridization. This technique may be very helpful for monoecious plants and for those which produce flowers in different periods and also to avoid barriers. Pollen bank can provide the viable pollen to commercial growers of fruits, vegetables and nut trees. Longevity of pollen, defined as the period of time over which the pollen retains its viability, *i.e.*, germination and fertilization, it varies greatly with plant species and storage conditions (Dafni & Firmage, 2000). There is a close correlation between longevity and moisture content of atmosphere to which pollen are exposed. Viable binucleate pollen could be stored for long period of time as compared to trinucleate pollen, the latter one lost viability rapidly (Hanna & Towill, 1995). Pollen of tomato stored in open air lost half of their original germination capacity within 2 days at 25°C and within 5 days at 6°C (Abdul-Baki, 1992), while pollen stored at –20°C under dry conditions retained viability for more than three years (Hanna & Towill, 1995). Aslantus & Pirlak (2002) reported that the germination capacity of strawberry pollen could be increased in low temperature. There are several reports on pollen germination and viability of different taxa with varied aims and objective like King (1961), Nair & Singh (1972), Mayer *et al.*, (1988), Shivanna & Rangaswamy (1992), Taylor & Hepler (1997), Thomas (2000), and Candace & Maureen (2003). Khan & Perveen (2006, 2009) and Perveen & Khan (2008) studied the germination capacity and viability of *Abelmoschus esculentus* L. (Malvaceae), *Mangifera indica* L. (Anacardiaceae) and *Malus pumila* L. (Rosaceae)

No reports are available on germination capacity and viability of stored pollen of this economically important plant from Pakistan. Present investigation is the first attempt to study the pollen germination ability of *Prunus amygdalus* Batsch.

Materials and Methods

Polliniferous material was collected in the flowering period of *Prunus amygdalus* from cultivated fields of Quetta and Khuzdar. Pollen of almond have been stored in different conditions as refrigerator, freezer and freeze drier but before that fresh pollen were systematically subjected to preliminary viability tests Alexander (1969). Pollen culture media was prepared according to standard method of Brewbaker & Kwack (1963). The germination was scored after 6 hours of incubation at room temperature in humid chambers using different solutions. Pollen tubes 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 slides were also prepared for light microscopy (LM) using the standard procedure of Erdtman (1952). Observations were made with a Nikon type- 2 microscope.

Results and Discussion

Pollen stored in freeze drier (–60ºC) showed better percentage of germination. The percentage of germination is 70.50% in 20% solution after 4 weeks and 50% germination was noted after 48 weeks with 40% solution (Table 1). This method seems to have more potential to maintain viability and germination as compared to other storing conditions *i.e.* refrigerator and freezer. Knowlton (1922) found 50%-80% relative humidity to be optimum for pollen of the species and varieties of *Prunus*, *Pyrus* and *Vitis*. Similarly, pollen stored in freezer at -20ºC and -30ºC showed good percentage of germination but as the time passed the percentage of germination gradually decreased and after 48 weeks the germination was 33.40% and 31.40 % respectively (Table 1). Weinbaum *et al*., (1984) exposed pollen grains of selected cultivar of almond and peach to a range of temperature between 1ºC-34ºC on a thermogradient plate, pollen germination at temperature below 9ºC was conspicuously greater in almond than peach., maximal germination percentages were attained at 16ºC in almond and at 23ºC in peach. The percentages of germination at 4ºC and fresh pollen was 58.40% and 63.40% after 4 weeks. Pollen stored at 4ºC showed above 60% germination in early weeks but then germination decreased and after 48 weeks the percentage of germination was 19.60% (Fig. 1). Not only the germination percentage of pollen stored at 4ºC drop sharply but the pollen tube growth was reduced it become more conspicuous with time, the color of pollen also faded. Singh (1961) observed pollen germination of almond in solutions of sucrose, lactose and sucrose plus agar at different concentrations. 10% agar solution was found to be the best concentration both for lactose and sucrose giving germination percentages 46.1 and 53.3 respectively. The germination was increased to 56.1 in sucrose solution by adding 1% agar. Porlingh (1956) studied pollen germination in 25 varieties of almond, apple, cherry, pear and plum in sucrose agar medium and found more than 80% germination except in cherries and triploid apples and pears. King & Hesse (1938) reported optimum temperature of 36ºF for 16 deciduous plants and 50% humidity is optimum for the pollen storage of different species and varieties of *Prunus*, *Pyrus* and *Vitis*.

Pollen of almond were also treated in vacuum over silica jell, this condition showed good germination up to 14 hours but decreased after that. Vacuum dried pollen showed better percentage of germination as compared to organic solvents.

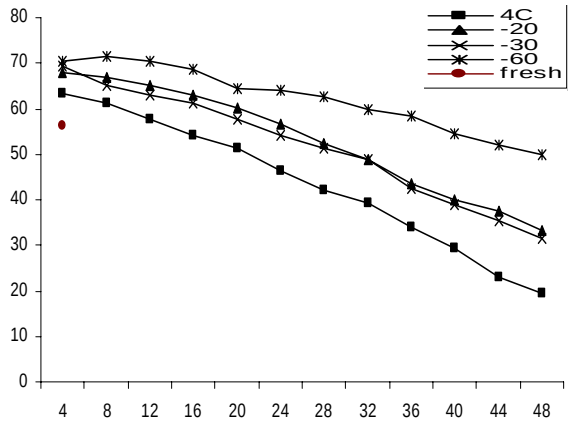

Fig. 1. Germination capacity of *Prunus amygdalus* pollen in different solutions of sucrose and boric acid.
Percentage of germination in fresh pollen at room temperature: 58.40%.

Table 1. Germination capacity of *Prunus amygdalus,* Pollen in different solutions of sucrose and boric acid.

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	63.40	20	68.00	20	69.30	20	70.50	20
8	61.30	20	67.00	20	65.10	20	71.60	20
12	57.60	20	65.00	20	63.00	20	70.50	20
16	54.00	20	63.00	20	61.10	30	68.70	20
20	51.30	30	60.10	20	57.60	20	64.50	20
24	46.30	10	56.70	30	54.00	30	64.00	20
28	42.00	20	52.50	20	51.20	30	62.60	30
32	39.40	20	49.00	20	48.70	30	60.00	20
36	34.00	20	43.50	20	42.60	40	58.30	20
40	29.50	20	40.00	30	39.00	20	54.60	20
44	23.00	20	37.50	30	35.40	20	52.00	40
48	19.60	20	33.40	40	31.40	20	50.00	40

Conclusion

Temperature and humidity are the major influencing factors of pollen behavior in different conditions. Pollen stored at freeze dryer (-60ºC) showed 50% of germination after 48 weeks. The most important factors for successful pollen conservation are storage temperature and moisture content of material; lowering both tend to increase the period of viability. Long-term storage has been achieved in many taxa by freeze-drying method Khan & Perveen (2006, 2008; Perveen & Khan, 2009).

Acknowledgement

We are thankful to PSF (Pakistan Science Foundation) for providing financial support.

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(Received for publication 23 June 2012)