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Assessment of pollen morphology, viability and germination in subtropical plum (*Prunus salicina* Lindl.) grown under *Tarai* region of Uttarakhand

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Abstract
The present investigation was carried out at Horticultural Research Centre (HRC), Patharchatta, GBPUA&T, Pantnagar to study the pollen morphology, viability and germination of pollen grains in two low chilled plum cultivars namely; Satluj Purple and Kala Amritsari. The pollen morphology (polar length and equatorial diameter) was measured through scanning electron microscope (SEM), while the pollen viability and germination were determined by acetocarmine and sucrose solution test, respectively. The results demonstrated that polar length and equatorial diameter was more in Kala Amritsari, while Satluj Purple was smaller in size; however, the polar/equatorial diameter (P/E) ratio was more under Satluj Purple. Moreover, the pollen viability and germination were higher in Satluj Purple as compared to Kala Amritsari.

Keywords: *Prunus salicina*, *Tarai*, Uttarakhand

Introduction
Plum (*Prunus* spp.) is one of the most important stone fruit crop of temperate region grown throughout the world. It belongs to the family Rosaceae and subfamily Pomoidae. Plum fruit is a rich source of vitamins and minerals and contributes significantly to human nutrition because of its richness in fiber and antioxidants (Kim *et al*., 2003) [5]. It has a high content of organic acids, such as neo-chlorogenic and chlorogenic acid, which also possess antioxidant properties (Heo *et al*., 2007) [4]. The two most common plum species grown throughout the world are European plum (*Prunus domestica* L.) and Japanese plum (*Prunus salicina* Lindl.). Japanese plums were first introduced to India by the European settlers and missionaries around 1870. In India, predominant plum growing areas are Himachal Pradesh, Jammu and Kashmir, Uttarakhand, Uttar Pradesh and Tamil Nadu. The annual production of plum in India accounts to 89 thousand metric tons from an area of 24 thousand hectare with an average productivity of 3.70 tonnes per hectares (Anonymous, 2019) [1]. Uttarakhand provides excellent climatic conditions for its production, with a total of 36 thousand metric tonnes production of plum from an area of 8.90 thousand hectares. In *Tarai* region of Uttarakhand, Japanese plum cultivars having low chilling requirements, particularly 'Satluj Purple', 'Kala Amritsari', 'Titron' and 'Aloo-Bukhara' are cultivated on small scale.

The crop reliability of fruit crops is highly dependent on genetically controlled mechanisms as well as external factors (Makovics-Zsohar and Halasz, 2016) [9]. Among the genetic control mechanisms, optimal realization of the cropping potential and knowledge of various aspects of the fertilization biology of fruit trees is essential (Cerovic and Micic, 1996) [3]. The genetic factors, which determine the success or failure of fertilization, include pollen genotype, pollen viability, germination of pollen into the stigma, pollen-pistil incompatibility reaction, and synchrony between pollen tube arrival to the ovule, embryo sac maturation and early embryo development (Rodrigo and Herrero, 1998) [11]. Pollen viability has been reported to vary from species to species and cultivars to cultivars depending on the ploidy level and the degree of hybridity of the cultivar. Kolesnikov (1966) [6] divided plum cultivars into three groups on the basis of pollen germination. These three groups consisted of best cultivars with 71 to 100 per cent, medium cultivars with 31 to 70 per cent germination and poor cultivars with 0 to 30 per cent germination. The only cultivars belonging to the first and second group were recommended as pollinizers. The present study was conducted to examine the morphology, viability and germination of pollen grains in two plum cultivars, which are commercially grown in the Tarai region of Uttarakhand.

Material and methods

The experiment was carried out at Horticultural Research Centre, Patharchatta, GBPUA&T Pantnagar (Uttarakhand) on two plum cultivars namely; Satluj Purple and Kala Amritsari. The anthers from selected flowers in both the cultivars were collected early in the morning with the help of forcep and needle, and kept in petridishes. For estimation of pollen morphology, pollen grains were mounted on the iron stabs by using double sided cello tape and allowed to dry for 24 hours. These stabs were kept inside the gold coater for gold coating for the conductivity of the pollen grains. Finally pollen grains along with the stab were kept inside the JEOL JSM-6610LV scanning electron microscope for examining pollen morphology. For determination of pollen viability, fresh pollen grains were dusted on slide and 1-2 drops of acetocarmine was added to the pollen mass. The slide was left as such for about 15-20 minutes to allow the pollen to absorb the stain completely. Several fields of pollen grains in different slides were examined under the microscope to count the pollen grains. Deeply stained and normal looking pollen grains were considered as viable ones, while shrivelled ones and weekly stained were regarded as non-viable. For pollen germination, pollen grains were placed in small drops of 10 per cent sucrose + 10 ppm boric acid solution on cavity slide at room temperature and covered with cover slips. The slides of both the cultivars were placed for germination in petri dishes containing a moist filter paper to ensure uniform and high relative humidity. The pollen germination was observed after 72 hours under four to five microscopic fields and germination percentage of both cultivars was worked out.

Result and Discussion

The data presented in table 1 clearly indicates that mean values of polar length and equatorial diameter differed in plum cvs. Satluj Purple and Kala Amritsari (Fig. 1). The pollen grains of cultivar Kala Amritsari were larger in size as compared to Satluj Purple. In the cultivar Satluj Purple, mean length of polar axis and equatorial diameter was measured 27.42µm and 18.54µm, respectively; whereas, polar length and equatorial diameter of pollen grains in Kala Amritsari was 29.13µm and 19.76µm, respectively. The polar/equatorial diameter (P/E) ratio of pollen grains varied from 1.46 in Kala Amritsari to 1.48 in Satluj Purple.

Table 1: Pollen morphology in plum cultivars Satluj Purple and Kala Amritsari

Cultivar	Polar length (µm)	Equatorial diameter (µm)	P/E Ratio
Satluj Purple	27.42	18.54	1.48
Kala Amritsari	29.13	19.76	1.46

Thakur (2013) [12] also observed significant variation in the size of pollen grains varying from 33.18µm to 43.46µm in length and 19.98 µm to 26.19µm in diameter in 8 cultivars of plum. The scanning electron microscopy (SEM) studies carried by Milatovic and Nikovik (2019) [10] in different plum cultivars revealed significant differences in morphology of different plum cultivars. Lal (1993) [8] reported the larger size of pollen grains in all the *domestica* cultivars as compared to *salicina* group of plum.

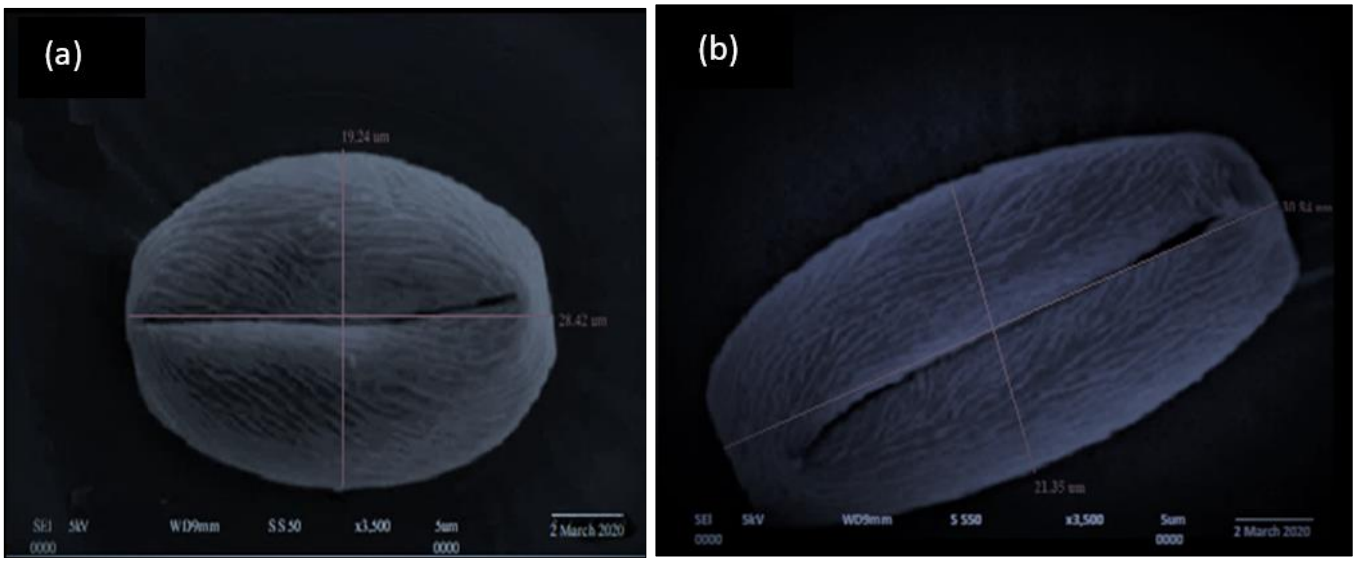

Fig 1: Polar length and equatorial diameter of pollen grains in plum cultivars (a) Satluj Purple, (b) Kala Amritsari

It is evident from the data presented in table 2 that pollen viability varied in both the cultivars. The cultivar Satluj Purple exhibited high pollen viability per cent (90.45%) as compared to Kala Amritsari (81.32%). The data on pollen germination showed the maximum pollen germination recorded in Satluj Purple (39.54%); whereas, Kala Amritsari had 36.76% per cent germination. Similar reports of high pollen viability and germination in Satluj Purple plum is reported by Kour (2018) [7]. Bal and Nandan (2013) [8] also reported highest pollen viability (97.4%) in Satluj Purple followed by Kala Amritsari (88.09%), among 9 cultivars of plum under investigation.

Table 8: Pollen viability and germination in plum cultivars Satluj Purple and Kala Amritsari

Cultivar	Pollen viability (%)	Pollen germination (%)
Satluj Purple	90.45	39.54
Kala Amritsari	81.32	36.76

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