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Pollinizer Influence on Fruit Quality Traits in Japanese Plum (*Prunus salicina* Lindl.)

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Abstract
Most Japanese plum-type cultivars are self-incompatible and therefore cross pollination is necessary to ensure fruit set. Moreover, pollen can directly affect fruit development process and quality. This study was carried out to investigate the effect of 6 pollen sources (‘Goje Sabz’, ‘Simka’, ‘Shablon’, ‘Methley’, ‘Myrobalan’ and ‘Black Star’) on fruit quantitative and qualitative characteristics of *Prunus salicina* cultivar ‘Black Star’, and identify the most effective pollinizer in the north region of Iran. The results showed that the pollen sources significantly affected different fruit characteristics such as fruit set, length, diameter, fresh weight, dry matter content and firmness. In contrast, total soluble solid, pH, total acid, vitamin C and total phenol did not significantly affect by pollen sources. Furthermore, it was found that the highest fruit set, physical dimensions, fresh weight and dry matter content were achieved when ‘Simka’ was used as pollen source. Finally, these results suggest that the pollen sources had xenia effects in Japanese plum and ‘Simka’ is the most suitable pollen source for Japanese plum ‘Black Star’ cultivar.

Keywords: fruit set, plum, pollination, self-incompatible.

Abbreviations: FW, fresh weight; **GAE**, gallic acid equivalent; **TSS**, total soluble solid; **TA**, titratable acidity.

Introduction

Plum is the most cultivated stone fruit in the world and has the second rank in the term of total production (Cuevas et al., 2016). The most commercial plums are classified intwo groups i) the hexaploid European plum (*Prunus domestica* L.) and ii) the diploid Japanese or Asian plum (*Prunus salicina* Lindl.) (Basanta et al., 2016). Cultivars of Japanese plum have wide variability in phenology, type and quality of fruit, vitamin C, phenolic composition, antioxidant activity and both skin and pulp color (González et al., 2016).

Japanese plums mostly consumed as fresh fruit, particularly because of having a number of health-promoting metabolite in which are useful for treating cardiovascular and cancer diseases. Fruit that do not qualify for the fresh fruit market owing to shape, size, colour, other quality attributes or overproduction could be used for producing plum beverages (de Beer et al., 2012). Cultivars of Japanese plum have higher flavor and aroma, better texture, more color, bigger size, and good nutritional values. Moreover they include reasonable source of fiber and proper source of bioactive compounds (such as vitamin C and phenolic composition) and antioxidant

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activity when compared to many other fruits (Fanning et al., 2014). Iran, with an annual production of 295000 tons, is in the fifth rank of plum producers in the world and 'Black Star' cultivar is one of the most suitable cultivars of Japanese plum in Iran (Pirkhezri et al., 2014).

Japanese plum, like other *Prunus* fruit tree species is self-incompatible and therefore it is necessary to have pollinizer trees in order to guarantee fruit set in commercial orchards (Guerra and Rodrigo, 2015). Reliable methods are required to determine the compatibility among different cultivars which include fruit set recording after controlled pollinations in orchard conditions (Guerra et al., 2009). Therefore, improvement of the fruit production is needed to exploit the variation among male cultivars in the germplasm collection with two strategies i) increasing the pollination efficiency by selection for pollen compatibility, and ii) selection for the direct influence of pollen on fruit size and fruit quality. Pollen source may affect the maternal tissues of the fruit or tissues surrounding embryo (Rezazadeh et al., 2013).

Pollen source is particularly important for self-incompatible cultivars. In self-incompatible cultivars, the consequence of poor pollination or self-pollination negatively influence the fruit set, size and sugar content (Wallace et al., 2002). Moreover, pollen directly affects fruit physical characteristics such as fruit size, weight, color, ripening time, quality and seed weight (Shafique et al., 2011). Effect of pollen source on fruit characteristics is known as xenia. Xenia is an immediate influence of foreign pollen on non-maternal tissue of the seed. Studies have shown the importance of this phenomenon in cross pollinated plants (Fattahi et al., 2014).

Although, Guerra et al. (2009) and Guerra et al. (2011) reported that fruit set of the Japanese plum significantly affected by different pollen sources, but there is no reliable information toward the effects of different pollen sources on fruit quality in Japanese plum cultivar 'Black Star'. Therefore, the objective of this study was to investigate the effects of pollen sources on quantitative and qualitative characteristics of Japanese plum cultivar 'Black Star'. Moreover, bioactive compounds (vitamin C and total phenolic content) of fruits and the best pollen source for pollination of this cultivar were investigated.

Materials and methods

Plant materials and treatment

This study was conducted in a commercial plum orchard in Daland, Golestan Province in northern Iran, during the 2015 growing season. Five days before flowering, at the balloon stage, branches with flower buds of pollinizers ['Goje Sabz' (or Greengage, *Prunus domestica* ssp. italica), 'Simka' (*P. salicina*), 'Shablon' (*P. salicina*), 'Methley' (is from a chance cross of *P. salicina* and *P. cerasifiera*), 'Myrobalan' (*P. cerasifera*) and 'Black Star' (*Prunus salicina*)] were cut off and were transferred immediately to the laboratory. All selected cultivars ('Simka', 'Shablon', 'Methley' and 'Black Star') and genotypes ('Goje Sabz' and 'Myrobalan') are the main *Prunus* species that generally are cultivated in Iran and have the best quality and acceptability.

The branches of pollinizers were kept in 20 ± 2 °C in water-sucrose solution for 5 days. After flowering, the pollens were obtained from flowers by removing and drying the anthers at room temperature during 24 h. The pollen was then sieved using a fine mesh (0.26 mm) and stored until further usage. Flowers of selected branches emasculated and covered by a paper bag to avoid self and/or cross pollination. Controlled pollination of isolated flowers was conducted in 50% of full bloom. Hand pollination was carried out 2 times at 3-day intervals. Pollen is placed on the stigma by finger touch, and covered again by a paper bag to prevent possible wind cross pollination.

Fruit set

The fruit set was calculated according to the following formula (Mazumdar and Majumder, 2003):

Fruit set (%) = (Number of fruits / Number of pollinated blossoms) × 100

Fruit physical and weight characteristics

Since self-pollinated trees produced no fruit, therefore fruit quantity and quality characteristics were determined based on 5 pollen sources (‘Goje Sabz’, ‘Simka’, ‘Shablon’, ‘Methley’, and ‘Myrobalan’). ‘Black Star’ plum fruits were harvested at firm-ripe stage. In each treatment, twenty fruits of uniform sizes that were disease-free and without other defects were selected to determine fruit quality. Physical dimensions of length and diameter of all fruits were measured by digital caliper. Fruits were weighed [fresh weight, (FW)] and dry matter (DM) was determined after 72 h drying (70 °C) using ventilation oven (Eraslan et al., 2007). Firmness was measured on both sides of the fruit after removal of the peel using a penetrometer (Reign Instrument GY-2, Co., Ltd.) fitted in 5 mm at tip (Mazumdar and Majumder, 2003).

- ***Total soluble solid, pH, and titratable acidity***

Total soluble solid (TSS) contents were determined with a digital refractometer (Euromex RD 635, Holland) to extract the juice of 10 fruits for each replication. Titratable acidity (TA), expressed as percentage of malic acid and determined by titration of 25 ml of filtered juice with the addition of 0.1 N NaOH solution to each juice extract to reach a pH of 8.2 at 20 °C (Mazumdar and Majumder, 2003).

- ***Vitamin C***

The vitamin C was determined by titration of 15 ml filtrated juice with 2, 6-dichlorophenol indophenol containing $NaHCO_3$. Ascorbic acid amounts were calculated using standard curve prepared from net ascorbic acid and finally expressed as mg ascorbic acid 100 g^{-1} FW (Mazumdar and Majumder, 2003).

Total phenolic content

Total phenolic content (TPC) was determined using the modified Folin–Ciocalteu method as described by Singleton et al. (1999). Polyphenol extraction was carried out with 10 ml acidic methanol added to 1 g specimen powder, kept at 4 °C, and filtered through ordinary filter paper. Next, 150 μl of this extract was diluted with 350 μl of distilled water, and then 2.5 ml of Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate were added to the mixture. This reaction solution was shaken in a shaker and kept in the dark for 2 h. The absorbance of the samples was measured at 765 nm with a UV/Vis spectrophotometer. Gallic acid was used as a standard sample for obtaining the calibration curve. Data were expressed based on milligram of gallic acid equivalent (mg GAE) per gram of fruit fresh weight.

Statistical analysis

The experiments were carried out according to a randomized complete block design (RCBD) with 4 replications (each replication contained 4 samples). Data were analyzed by PROC ANOVA procedure by SAS software (Ver. 9.1 2002–2003, SAS Institute, Cary, NC). Before analysis of variance, data were tested for normality using the Kolmogorov–Smirnov and Cochran tests, respectively. Tukey’s HSD test at $P \leq 0.05$ was calculated to compare differences between means following a significant ANOVA effect.

Results and discussion

Fruit set

It was revealed that fruit setting of pollinated Japanese plum cultivar ‘Black Star’ was significantly affected by pollen sources. As fig. 1 has shown, fruit setting of ‘Black Star’ ranged between 19.93% (by ‘Goje Sabz’) and 54.06% (by ‘Simka’) with cross-pollination however by self-pollination, no fruit setting was observed.

It was revealed that in many *Prunus* species, genotype and genotype interactions had important effects on the successful fruit setting and subsequent fruit yield (Jia et al., 2008). The variation in fruit set could be attributed to the variation in pollen viability, pollen transfer to the stigma, pollen-pistil incompatibility reaction, synchrony between pollen tube arrival to the ovule and embryo sac maturation, fertilization of at least one ovule, and successful early embryo development (Guerra et al., 2011; Rezazadeh et al., 2013). Moreover, the lack of fruit setting in self-pollination could be explained by self-incompatibility (Sapir et al., 2007).

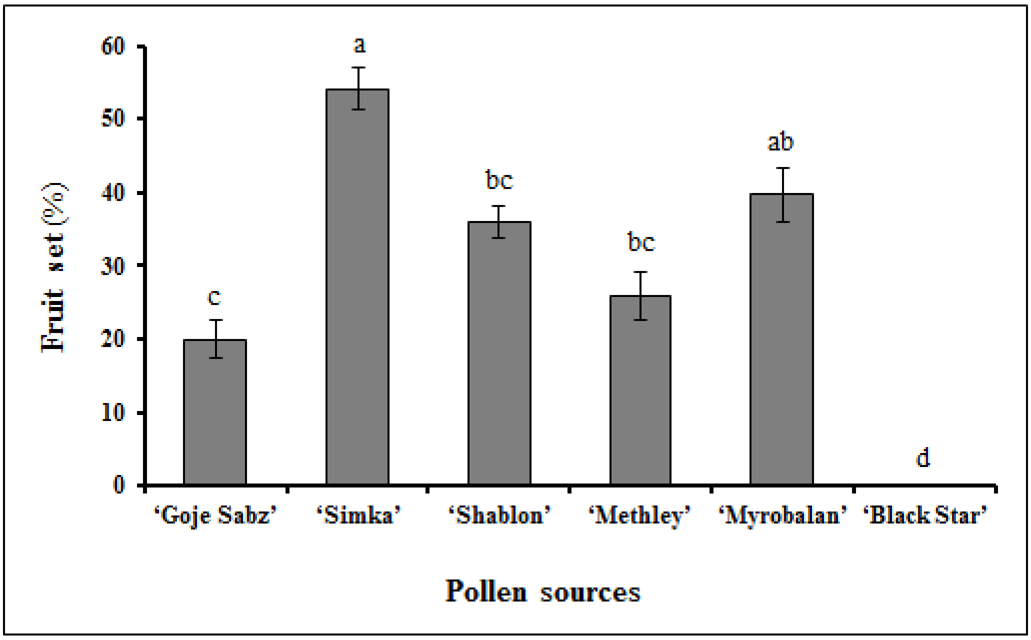

Fig. 1. Effect of different pollen sources on fruit set of Japanese plum cultivar 'Black Star'. The values are the means (n = 4) ± standard error. Different letters indicate significant difference ($P \leq 0.05$) in the pollen viability (upper case) and fruit set (lower case) according to the Tukey's HSD test.

Fruit physical and weight characteristics

According to the fact that self-pollinated trees produced no fruit, therefore fruit quantity and quality characteristics were determined based on 5 pollen sources. Significant differences among the pollen sources were found in fruit length and diameter. Similar patterns were found in fruit length and diameter in response to different pollen sources, as the highest fruit length and diameter (52.48 and 59.25 mm) were obtained when 'Simka' was used as pollen source, that had no significantly difference with 'Myrobalan' genotype and 'Methley' cultivar (Fig. 2). Furthermore, 'Goje Sabz' genotype and 'Shablon' cultivar had the lowest fruit length and diameter when compared with other cultivars.

Our results are in consistent with Al-Khalifah (2006) and Rezazadeh et al. (2013)indicated that fruit physical dimensions were affected by pollen sources. These effects are related to xenia processes. Xenia results in changes in the size, shape, color, developmental timing, and chemical compounds of seeds and fruits found as a result of fertilization by different pollens. The term xenia was originally coined to describe such pollen effects only on maternal plant tissues, that is, on seed coats, pericarp, and attending structures (Denney, 1992; Kumar and Das, 1996).

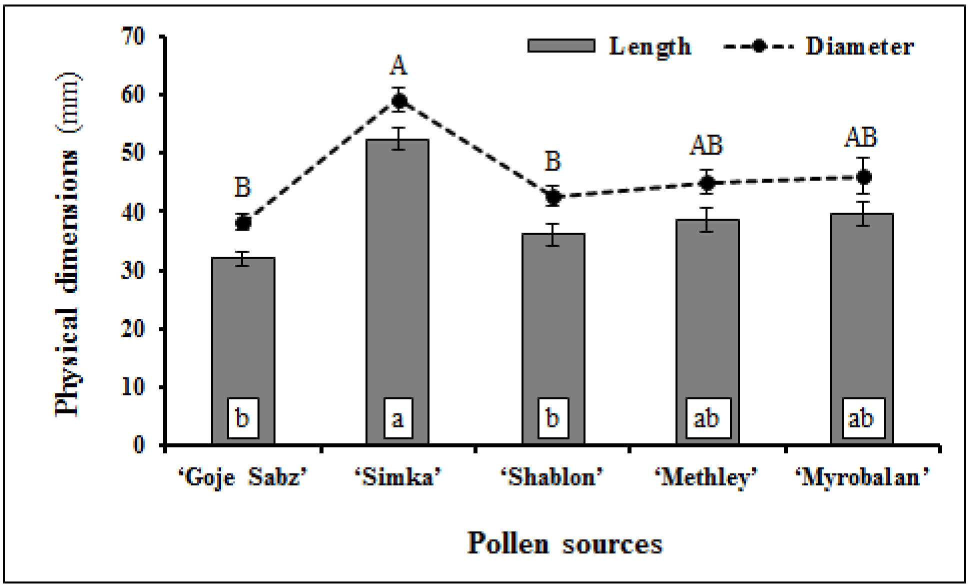

Fig. 2. Effect of different pollen sources on fruit physical dimensions (length and diameter) of Japanese plum cultivar 'Black Star'. The values are the means (n = 4) ± standard error. Different letters indicate significant difference (P ≤ 0.05) in the diameter (upper case) and length (lower case) according to the Tukey's HSD test.

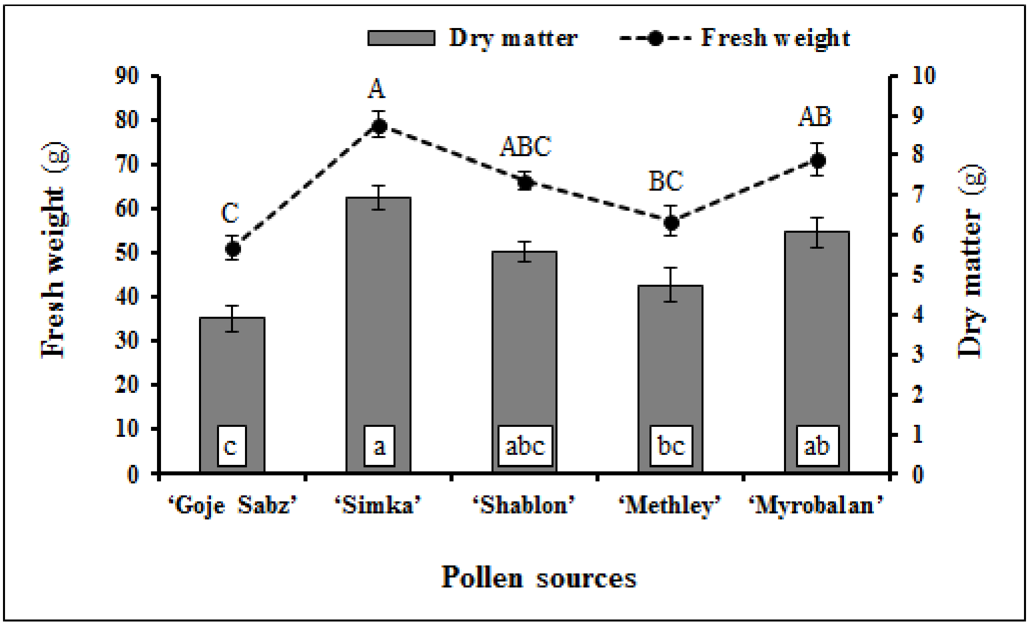

Fig. 3. Effect of different pollen sources on fruit fresh weight and dry matter content of Japanese plum cultivar 'Black Star'. The values are the means (n = 4) ± standard error. Different letters indicate significant difference (P ≤ 0.05) in the fresh weight (upper case) and dry matter (lower case) according to the Tukey's HSD test.

It was reported that specific pollens are capable of increasing indole-3-acetic acid (IAA) that possibly can affect the cell number in early fruit developmental stage and stimulate cell elongation (El-Hamady et al., 2010; Khan and Chaudhry, 2010). Rezazadeh et al. (2013) suggested that the variation in physical traits of fruits in response to different pollen sources could be due to the variations in yield depending mostly on fruit set. Additionally, it was reported that pollen sources changed fruit physical dimensions which could be due to the specific pollens affect the cell number in early fruit development (El-Hamady et al., 2010). However, Javadi and Gheshlaghi (2006) reported that pollen source had no effect on fruit size of hazelnut that could be as a result of unsuitable pollinizer (Hossein-Ava et al., 2006).

Fruit fresh weight (FW) and dry matter (DM) content of Japanese plum cultivar 'Black Star' significantly affected by different pollen sources. The similar pattern was obtained in FW and DM by pollination by different pollinizers (Fig. 3). Among pollinizers, 'Simka' had the highest FW and DM content (78.98 and 6.93 g, respectively) as compared with others. Moreover, 'Goje Sabz' genotype produced fruits with the lowest FW and DM content (19.93 and 3.90 g, respectively).

Al-Khalifah (2006), Hossein-Ava et al. (2006), Pritchard and Edwards (2006), Gaaliche et al. (2011), Seal et al. (2013) and Rezazadeh et al. (2013) reported that pollen sources significantly affected fruit fresh and dry weight, which is in consistent with current finding. Pollen source affects has been reported on growth of ovarian tissues-by stimulation of hormone production, and specific effect on the fruit growth as well as fruit fresh and dry weights (Shafique et al., 2011). The variation of fruit weight in response to different pollen sources could be related to the effect of specific pollens on the cell division and subsequently cell number in early fruit development (El-Hamady et al., 2010).

As shown in Table 1, fruit firmness was also significantly influenced by different pollen sources. Fruits obtained from trees that pollinated by 'Shablon' (6.18 N), 'Goje Sabz' (6.00 N), 'Simka' (5.87 N) and 'Methley' (5.75 N) had the highest fruit firmness respectively, while the lowest fruit firmness was observed in 'Myrobalan' genotype (4.50 N).

This variation might indicate earlier maturation or crisper pulp (El-Hamady et al., 2010). In agreement our findings, previous studies reported that pollen sources potentially affect fruit tissue properties as well as firmness (Seal et al., 2013; Militaru et al., 2015). However, the effect of different pollen on fruit tissue quality might be because of differential efficacy of the pollen sources to fertilize a number of ovules (Jalikop and Kumar, 2007).

TSS, pH and TA Fruit quality parameters such as TSS, pH and TA are not significantly affected by pollen sources. The highest TSS, pH and TA was found in 'Myrobalan' (10.10 °Brix), 'Goje Sabz' (3.69) and 'Simka' (2.16 %), respectively, but significant difference was not observed when compared to the other cultivars.

Our results are in agreement with Kahn et al. (1994) and Allah Tavakoli et al. (2014), who concluded that pollen of parent has not significant effect on fruit quality characteristics such as TSS and TA content.

Table 1. The effects of pollen sources on some fruit quality characteristics of Japanese plum cultivar 'Black Star'.

Pollinizers	Firmness (N) *	TSS (°Brix) ns	pH ns	TA (%) ns	Vitamin C (mg $100g^{-1}$ FW) ns	TPC (GAE $100g^{-1}$ FW) ns
'Goje Sabz'	6.00 a†	9.03 a	3.69 a	2.06 a	3.44 a	137.50 a
'Simka'	5.87 a	9.77 a	3.67 a	2.16 a	3.25 a	151.56 a
'Shablon'	6.18 a	9.99 a	3.63 a	2.12 a	3.23 a	148.75 a
'Methley'	5.75 a	9.88 a	3.46 a	2.00 a	3.26 a	139.37 a
'Myrobalan'	4.50 b	10.10 a	3.57 a	2.12 a	3.56 a	137.50 a

* and ns indicates significant at P ≤ 0.05 and non-significant, respectively.

† For each characteristic, means followed by the same letters are not significantly different at P ≤ 0.05 according to Tukey's HSD test. TSS, total soluble solid; TA, titratable acidity; TPC, total phenolic content.

Bioactive compounds

No significant differences were observed between the pollen sources and vitamin C content in Japanese plum cultivar 'Black Star' (Table 1). The highest vitamin C content was obtained in fruits that pollinated with 'Myrobalan' genotype (3.56 mg $100g^{-1}$ FW), however no significant difference was obtained with other pollinizers. Similar to our results El-Hamady et al. (2010) reported that pollen sources had not significant effect on vitamin C content of date palm (*Phoenix dactylifera* L.)

Although there was no significant difference among the pollen sources in TPC, results showed that the highest fruit TPC (151.56 GAE $100g^{-1}$ FW) was achieved when 'Simka' was used as pollen source, followed by 'Shablon', 'Methley', 'Myrobalan' and 'Goje Sabz' (Table 1).

These results are in accordance with El-Sharabasy and El-Banna (2009) and Shafique et al. (2011). They confirmed that pollen source did not significantly affect the level of total phenols in the palm fruit (Shafique et al., 2011). Moreover, it has been revealed that carbohydrates that are generated during photosynthesis form the building blocks for all other carbon-based compounds in the cell, including the phenolic compounds (Vermerris and Nicholson, 2006). In fact, the specific pollen containing various amounts of carbohydrates (Shivanna, 2003), can also affect the total phenolic compounds biosynthesis by transferring these substances to the endosperm and embryo tissues and results in the more phenolic productions.

Conclusion

The selection of the pollen source had a potential to modify and improve Japanese plum (*Prunus salicina* Lindl.) production. The effects of pollen sources on fruit quantitative and qualitative characteristics are known to occur in several plum cultivars and genotypes that confirmed the xenia effect in our study. These differences have been attributed to the positive effect of cross-pollination. Our results confirmed that pollen cross-parents in Japanese plum cultivar 'Black Star', play significant role on physical characteristics of the fruit, however positive effect was not observed on qualitative characteristics. Results related to the efficiency of six pollen sources suggest application of 'Simka' as pollen source for cross-pollination of 'Black Star' cultivar.

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