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The Feasibility Study on the Prediction of Ploidy Levels Base on Pollen Dimensions in *Rosa*

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Abstract

Ploidy level is one of important factors for plant breeders; therefore models that can predict it are very practical. To estimate of ploidy levels in some species, hybrids and cultivars of *Rosa*, dimensions of over 500 pollens include pollen length, width and area measured at ABRII. The calculations were carried out in several stages. First, Ploidy levels were regressed with pollen dimensions, after that, pollen area was regressed with pollen length and width. Finally the best models from previous step were tested to estimate of pollen level. In the present study, although a highly positive correlation ($r^2 \geq 0.80$, except for 2x) was obtained between pollen length and pollen area, but to predict of ploidy level, the correlation between it and pollen demotions was very weak ($r^2 \geq 0.24$). Results of test the best models (with highest r^2 and lowest SES) for estimating of ploidy level, despite could be used, but because of the strong correlation among them, were not reliable.

Keywords: Estimation, Ploidy level, Pollen, *Rosa*.

INTRODUCTION

Roses are appreciated all over the world for their beauty and scent, and are by far the best selling cut flowers worldwide. Chromosome numbers in the genus Rosa are based on multiples of seven and range from 2n=2x=14 to 2n=8x=56 (Darlington and Wylie, 1955). Most species are diploid, whereas most of the modern rose cultivars are tetraploid and usually interfertile (2n=4x=28), (Smulders *et al.*, 2011).

In the four sub-genera of Rosa, the three sub-genra (*Hulthemia, Platyrhodon* and *Hesperodos*) each contain one species in which 2n=2x. In the fourth subgenus, (*Eurosa*), 120 species are divided in 10 sections. In the sections, there are different ploidy levels include 2n=2x, 4x, 5x, 6x and 8x (Darlington and Wylie, 1955).

Modern rose cultivars have a narrow genetic background. Species that contributed to the genepool are mostly diploid, some are tetraploid. Modern roses are grouped into horticultural classes that include: Polyanthas (2n=2x), Hybrid Teas (2n=3x, 4x), Floribundas (2n=3x, 4x) and Miniatures (2n=2x, 3x or 4x) (Yokoya *et al.*, 2000).

For plant breeders, ploidy level is an important consideration because it can influence male and female fertility, cross fertility, plant vigor and gene expression (Contreras *et al*., 2007; Ranney, 2006). Ploidy level can have a profound influence on plant phenotype, physiology, environmental adaptation, pest susceptibility, fertility, and mating success (Levin, 2002) and likely contributes to the wide geographical and climatic adaptation of roses. Ploidy characterization of individuals and populations can be very useful to better understand population structure, gene flow, and develop effective and efficient breeding strategies.

One of methods to determine of ploidy level in plants is direct chromosome counts. The method requires individuals with specialized cytological skills and can be a tedious and time consuming process (Zlesak *et al*., 2005). This has led some rose researchers to explore alternative, indirect methods of ploidy assessment including flow cytometry, stomata or guard cell size, and pollen diameter (Semeniuk and Arisumi, 1968; Jacob *et al.*, 1996; Yokoya *et al*., 2000; Kermani *et al*., 2003; Zlesak *et al*., 2005; Joly *et al.*, 2006). Flow cytometry using macerated leaf tissue has become common for saprophytic ploidy characterization in recent rose literature (Kermani *et al.,* 2003; Leus, 2005).

Pollen size as well as pollen morphology are known to vary across genera dye to factors including genetic background, chromosome number, pollen maturity, location in the inflorescence, time of pollen grain development during flowering season, temperature, nutrition, and moisture conditions (Stanley and Linskins, 1974). Pollen diameter can be useful to estimate sporophytic ploidy level in many genera, aiding in species identification, polyploidization studies, and germplasm characterization for breeding and other purposes (Jacob and Pierret, 2000; Zlesak *et al.,* 2005).

Since the flow cytometry is a partly expensive technology and some of academic and research centers in Iran do not have access to it, the pollen dimensions can be a useful predictor of sporophytic ploidy depending on the germplasm and degree of accuracy that is needed. Pollen dimensions (diameter) have been proposed as a useful tool for sporophytic ploidy prediction in rose by Jacob and Pierret (2000), and Zelsak (2009).

Although some methods are available to determine ploidy levels for numerous plants, there is no information about estimation of ploidy levels for native Rosa in Iran. Therefore, the objective of present study was to predict and develop functions between pollen sizes and ploidy levels of some species, hybrids and cultivars of native Rosa in Iran.

MATERIALS AND METHODS

Plant material

We selected five popular roses cultivar with different ploidy levels from Germplasm Collection at the Agricultural Biotechnology Research Institute of Iran (ABRII). The details of plant material used for the present study are given in Table 1. Ploidy status of all these materials like species, ploidy level and source was confirmed.

Pollen size measurement

Open rose flowers of di-, tri-, tetra-, panta- and hexaploid plants were harvested during the flowering season. The ploidy levels of these plants were assessed by flow cytometer with a PARTEC Ploidy Analyzer PA (PARTEC II, Germany). Petals were removed, and the anthers were dried 2 hours in a Petri dish at ambient temperature. The pollen grains were not wetted for observation. Wetting of the pollen results in swelling of the pollen. The ellipsoid pollens becomes round through absorption of water. Observations were made with Nikon TE300 microscope equipment with camera. A magnification of 200X was used on a microscope. The smallest and largest diameter of 100 pollens was measured for each species or cultivar (a total of 522 pollens) by use of a Micro Measure 3.3, (Fig. 1).

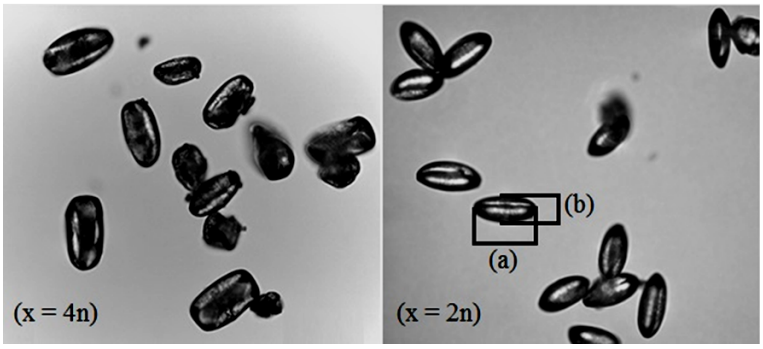

Fig 1. Pollen size in Rosa flowers of di- and tetrapploid, a and b are the largest and smallest diameter, respectively.

Area of per ellipse was calculated by following equation: ($A = \pi \times a \times b$), A is area of ellipse, a and b are semi-major and semi-minor radius, respectively.

The stages of calculations

First, ploidy level as a dependent variable was regressed with pollen length, pollen width and pollen area as independent variables in all samples.

Second, the dependent variable (pollen area) was regressed with independent variables, including pollen length and pollen width for use in models, estimating pollen area of *Rosa* with different ploidy levels.

In the next step, 20% of the total data that had not been previously operated in modeling were used for model validation. Standard error of the estimate (SES) and the values of the coefficients (b) and constants (a) were also reported. The estimated pollen area was determined by fitting the equation and eventually, validated model was selected based on the combination of the highest coefficient of determination (r^2) and the lowest SES.

Finally, about 20% of all data on five ploidy levels were tested in selected models to confirm the effectiveness of models for predicting of ploidy levels in *Rosa*.

Analysis was conducted in SPSS software ver. 23 and graphs were made, using Microsoft Excel. 2007.

RESULTS

The mean pollen length, width and area and also standard deviation and number of sampling at different ploidy levels in Rosa species, hybrid and cultivars were calculated and the data are presented in Table 2.

Table 1. Characteristics of plant material in different *Rosa.*

Species/Variety/cv	Ploidy level	Origin/Source
Rosa persica	2x	Germplasm Collection of ABRII* (Native of Iran)
Rosa hybrida cv. Ice Berg	3x	Germplasm Collection at ABRII
Rosa hybrida cv. Appollo	4x	Germplasm Collection at ABRII
Rosa canina	5x	Germplasm Collection at ABRII (Native of Iran)
Rosa hybrida cv. Abrii	6x	Germplasm Collection at ABRII

* ABRII is abbreviation of Agricultural Biotechnology Research Institute of Iran

Table 2. Characteristics of pollen diameter in different poloidy level in *Rosa.*

Poloidy level	Pollen width (µm)			Pollen width (µm)			Pollen area (µm²)		
	n	Mean	Stdev	n	Mean	Stdev	N	Mean	Stdev
2x	100	41.19	5.14	100	21.11	2.63	100	683.14	116.84
3x	106	36.64	8.77	106	17.72	2.59	106	518.23	163.16
4x	106	39.66	6.86	106	23.71	3.94	106	746.49	199.97
5x	100	31.35	10.00	100	17.60	3.99	100	456.31	211.43
6x	110	43.92	9.92	110	26.07	6.84	110	924.36	331.30

Relationship of ploidy level to pollen dimensions

The polynomial model produced the highest coefficient of determination (r^2) than other models (linear, power, exponential and logarithmic) that was equal to 0.14, 0.11 and 0.24, using pollen length, pollen width and pollen area, respectively (Fig. 2).

Relationship of pollen area to pollen length and width

In all ploidy levels, it was seen that pollen length versus pollen area in comparison with pollen width versus pollen area, produced a stronger correlation (in 6x, model tested

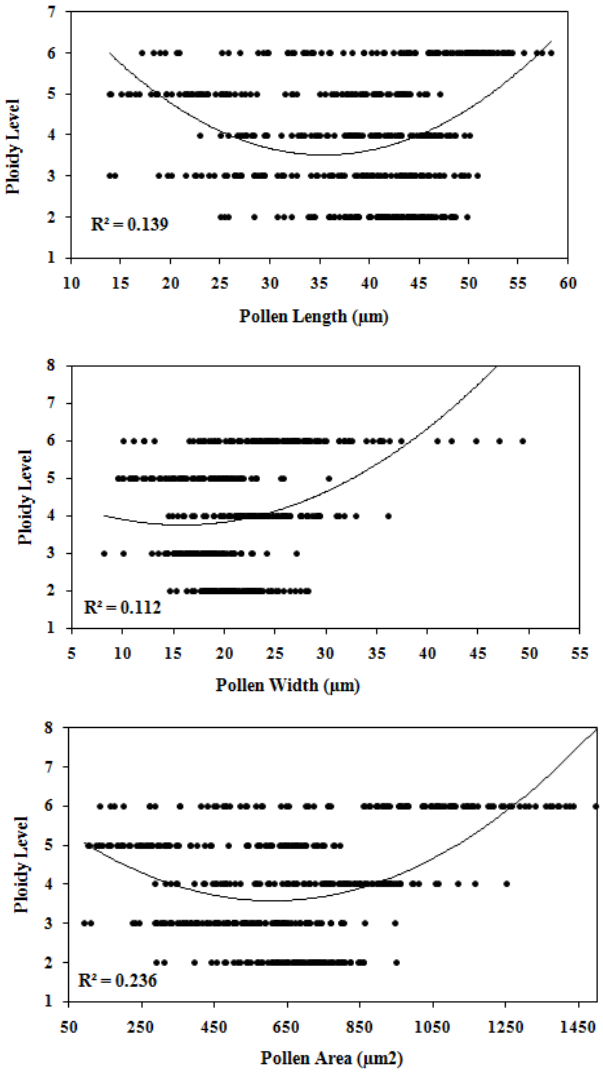

Fig. 2. Relationship between poloidy level and pollen length, width and are from Rosa species, hybrids and cultivars.

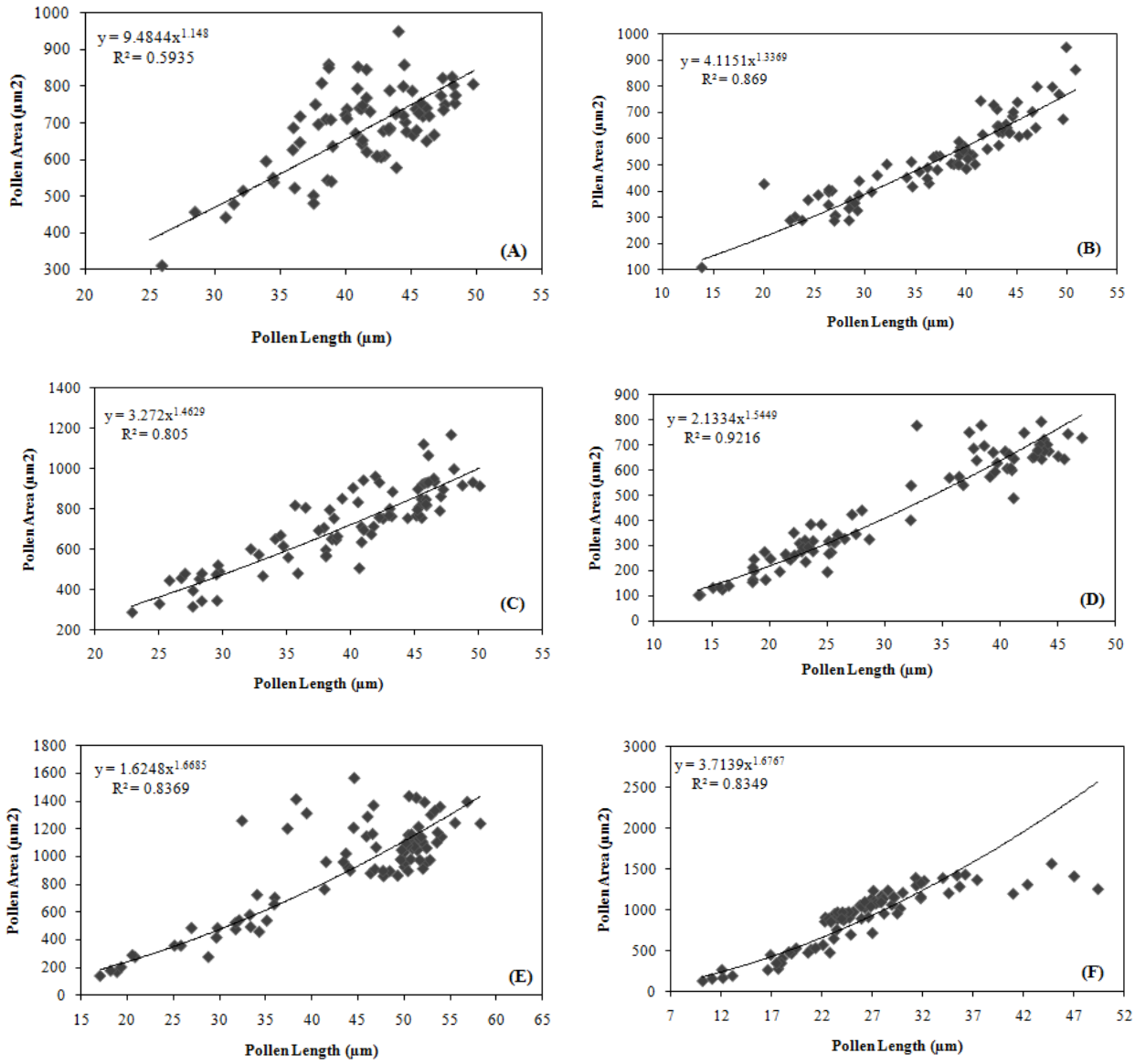

Fig. 3. Relationship between pollen area and pollen length from Rosa species, hybrids and cultivars.

base on pollen length and also pollen width were acceptable). Based on selection criteria previously described (higher R^2 and lower c) we select the best model for estimation (Table 2). Except for 2x, all ploidy levels produced a coefficient of determination (r^2) equal to or greater than 0.8. In all ploidy levels, the highest r^2 and lowest SES were obtained in power function (model) (Fig. 3).

Model validation

To validate the model, in per ploidy level, pollen area was predicted using the best model or equation ($y= ax^b$, where y is pollen area, x is pollen length and a, b are constants) base on pollen length measurements from the calibration experiment and was compared with the actual pollen area. Regression analyses were conducted. The pollen area was estimated by the power model strongly agreed with the measured values of pollen area, it is evident from higher value of r^2=0.92 and r^2=0.96 for 3x and 5x respectively. Estimation of pollen area using power model is partly acceptable in 2n and 6x with r^2=0.74 and r^2=0.7, respectively (Fig. 4).

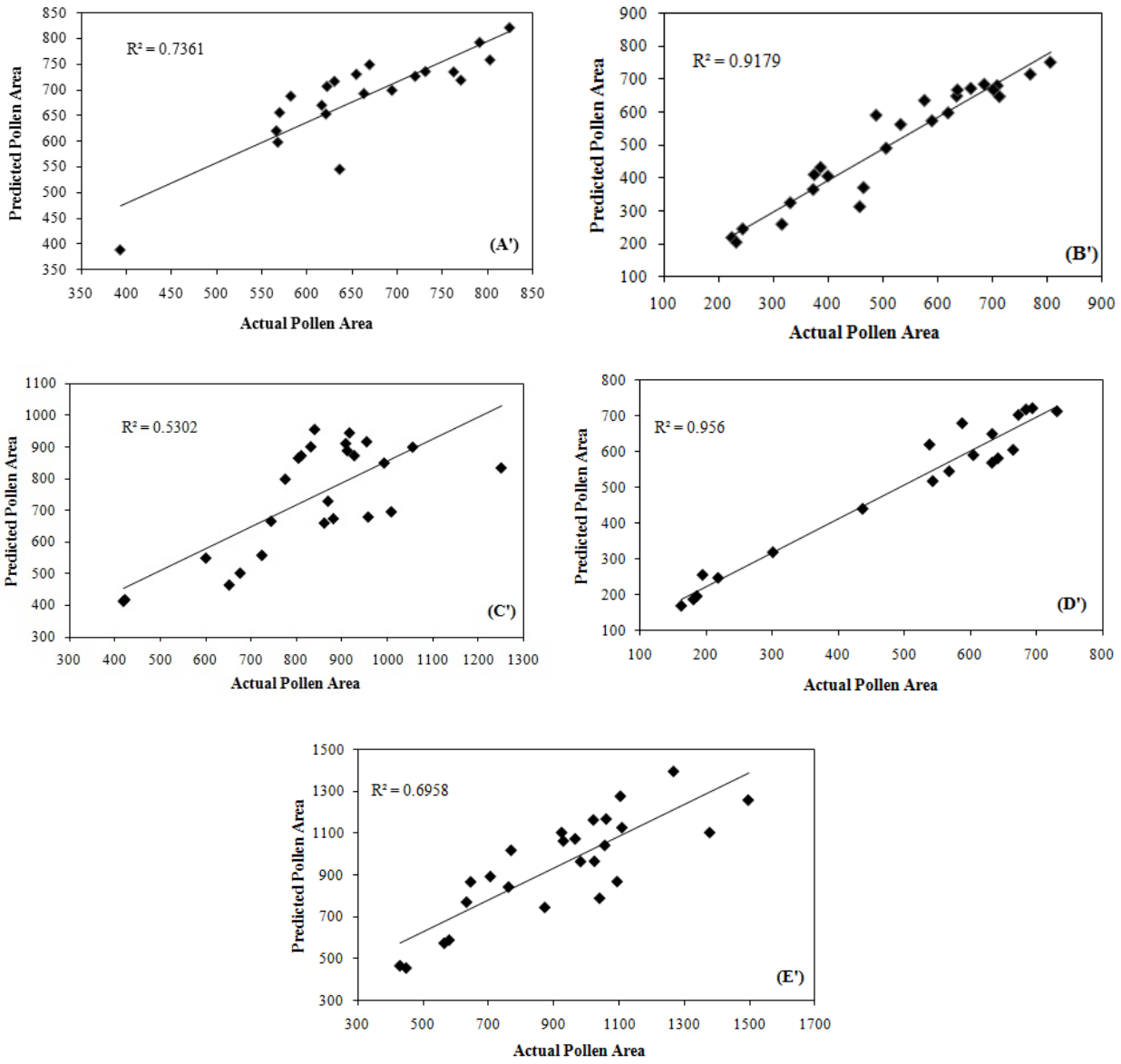

Fig. 4. Comparison of actual and predicted pollen area from Rosa species, hybrids and cultivars.

Test of selected models to ploidy level estimate

After that the best model or equation was determined for per ploidy level, the models were tested in 20% of actual pollen area for other ploidy levels, for examples, the best model for 2x ($y=16.28x^{12.46}$) was tested for 3x, 4x, 5x and 6x (Tabel 3).

The results of the test showed that in all ploidy levels except 2x, there was a high correlation between actual and predicted pollen area (r^2 was equal to or greater than 0.81%). That is, pollen area model (equation) of per ploidy level is applicable for other ploidy level; therefore ploidy levels were not found different in terms of the pollen area.

DISCUSSION

Our analysis yields three principal results. First, there is a weak correlation between ploidy level and pollen dimensions (pollen length, width and area) in these five species, hybrids and cultivars of *Rosa*, so that the ploidy level is not predictable by the models. A similar result has also been reported in rose collection of France (Jacob and Pierret, 2000), by Mishra (1997) in *Coffea*, between ploidy level and stomatal characteristics, Jones *et al.* (2007) between genome size and ploidy level in *Rhododendron* and *Padoan et al.*, (2013) in *Citrus* between ploidy level and some morphological traits. Studies showed generally the pollen size increases with ploidy level (Johan-

Table 3. Estimated regression coefficients (a,b), coefficient of determination (r^2), Standard error of the estimate (SES) values for predicting pollen area in different poloidy level in rose.

Poloidy level	Form of model tested	Pollen area vs. length				Pollen area vs. width			
		a	b	r^2	SES	a	b	r^2	SES
2n	Linear	16.28	12.46	0.49	86.54	32.28	1.76	0.57	80.00
	Logarithmic	634.92	-1671.99	0.52	83.71	704.10	-1457.93	0.60	77.09
	Power	1.15	9.48	0.59	0.13	1.16	19.70	0.56	0.13
	Exponential	0.29	202.31	0.54	0.14	0.50	222.07	0.51	0.14
3x	Linear	17.54	-126.15	0.86	60.62	43.48	-249.29	0.53	110.41
	Logarithmic	540.01	-1410.50	0.81	70.70	724.64	-1552.95	0.56	107.78
	Power	1.34	4.11	0.87	0.14	1.89	2.16	0.67	0.22
	Exponential	0.41	108.08	0.82	0.16	0.11	73.43	0.57	0.25
4x	Linear	25.09	-269.27	0.77	96.00	46.78	-353.55	0.73	103.58
	Logarithmic	905.13	-2590.42	0.76	97.24	1035.67	-2511.77	0.73	103.23
	Power	1.46	3.27	0.80	0.14	1.63	4.24	0.73	0.16
	Exponential	0.04	142.09	0.79	0.14	0.70	131.23	0.70	0.17
5x	Linear	20.04	.170.44	0.90	66.31	45.87	-357.41	0.77	102.53
	Logarithmic	577.62	-1499.45	0.90	67.89	750.70	-1681.58	0.76	104.56
	Power	1.54	2.13	0.92	0.16	2.09	1.05	0.84	0.22
	Exponential	0.05	78.08	0.88	0.20	0.12	44.50	0.80	0.25
6x	Linear	27.34	-269.61	0.69	194.35	41.37	-158.24	0.76	171.08
	Logarithmic	985.17	-2755.37	0.70	190.32	1081.22	-2561.51	0.84	140.93
	Power	1.67	1.62	0.84	0.22	1.68	3.71	0.83	0.22
	Exponential	0.04	115.87	0.78	0.26	0.60	170.77	0.67	0.31

son and van Bothmer, 1994), also it was partly, observed in our study.

Second, pollen area in *Rosa* of di, tri, tetra, panta and hexaploid is predictable with only measurement of one variable (pollen length).

Although in this study prediction of pollen area was calculated with high accuracy, but estimation of the variable is not so important, practically. Therefore in the third stage, the feasibility of ploidy level prediction base on pollen area was tested. Result of current study showed that using power model (equation) obtained from 3x, 4x, 5x and 6x, just in *Rosa* of diploid (here, *Rosa persica*), can estimate ploidy level but it is not so reliable, because there is about 0.3 positive correlation between them and it is not close to zero; therefore the estimation is associated with a high percentage of error. In other hybrids and cultivars, also there is not possibility for predicting of ploidy level, because a strong correlation was found between them.

It is concluded that to prediction of pollen ploidy in rose, if for any reason (breeding, etc.) there was a need to have pollen area and we have not much time for many measurements, with only measurement of pollen length, we can estimate it. For estimation ploidy level in species, hybrids and cultivars of Rosa the method mentioned above cannot be used reliably.

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