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POLLEN ANALYSIS IN SOME TULIP CULTIVARS

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Abstract

Viability and germination capacity of pollen grains is very important in breeding programs which require a minimum 30% germination level for the success of artificial hybridization. This paper presents the pollen viability and germination capacity analyses performed in nine tulip cultivars that belong to six different groups and the correlation between these two determinations. The viability of the pollen grains registered higher values in eight, out of nine cultivars ('Yokohama' - 81.7%, 'Veronique Sanson' - 93.9%, 'Tender Whisper' - 90.7%, 'Paul Scherer' - 86.0%, 'Davenport' - 84.8%, 'Fancy Frills' - 92.9%, 'Blue Parrot' - 96.1%, 'Red Riding Hood' - 89.8%), while in the case of germination capacity only four cultivars ('Red Riding Hood' - 35.9%, 'Blue Parrot' - 81.1%, 'Davenport' - 78.3% and 'Salmon Impression' - 27.7%) registered the necessary rate for the breeding programs. The lower germination percentage of pollen grains, in some cultivars, may be due to the duration of storage period of the pollen.

Key words*: breeding, pollen grain germination, pollen grain viability, tulips.*

INTRODUCTION

Although there are thousands of tulip cultivars over the world, breeders do not cease studying and creating new ones in order to satisfy the need for beauty of all mankind. Tulips, along with daffodils, hyacinths, gladioli are the most widely cultivated bulbous spring plants used to decorate green areas and also as cut flower in bouquets and other floral arrangements.

The study of germination capacity of pollen grains is very important in the selection of genitors and in a successful artificial hybridization, where its level should be at least 30% (Cordea, 2014). Germination capacity of pollen grains may be determined indirectly by testing the pollen viability and directly, by pollen germination *in vitro* on solid nutrient medium. Viability of pollen grains may be revealed with simple colouring tests (with potassium iodide or carmine staining method). It is well known that there is a very strong direct, positive correlation between germination and viability of pollen grains (Cordea, 2014).

The objective of this study was to evaluate the pollen germination and viability in nine tulip cultivars belonging to six classification groups in order to be used in breeding programs.

MATERIALS AND METHODS

Nine tulip cultivars belonging to six classification groups were tested in order to determine the germination capacity and viability of pollen grains (Brickell and Zuk, 1997; Raamsdonk and de Vries, 1996) (Table 1), tests having been performed in the Plant breeding laboratory of the Advanced Horticultural Research Institute of Transylvania, UASVM Cluj-Napoca.

Determination of germination

Germination capacity of pollen grains presumes that the fertile grains with viable cytoplasm and spermatia germinate and release pollen tube on an artificial nutritional medium enhanced with different sucrose concentrations (10-30%), with 70-90% humidity at a temperature between 20-22°C. There are different opinions on the concentration of the two ingredients which form the nutritional medium. Soares et al. (2013) has obtained the best results on a

medium with 15% sucrose and 0.8% agar in *Passiflora* sp. Some authors (Chagas et al., 2008) observe that higher concentrations of agar increase the germinated pollen grain ratio in pear cultivars while others (Luza and Polito, 1985) came to the conclusion that in nut cultivars a relative high concentration of agar inhibits pollen germination and decreases the pollen tube length.

Our tests were performed on solid nutritional medium according to the protocol described by Cordea (2014). In this regard anthers were collected from unopened flower buds before the pollen has reached maturity. The anthers have been placed on Petri dishes and maintained for a period of 48-72 hours in room temperature (22^{o}C) until they broke and released the pollen grains. The dried pollen grains have been sown on solid medium made of 1.5% agar and 15% sucrose with 85% humidity, preserved at 22^{o}C temperature.

The microscope readings have been performed in 24 hours after sowing the pollen grains on the nutritional medium. In order to obtain a precise counting of the examined grains there were chosen certain uncrowded microscopic fields and observations have been made with the 4x and 10x lenses (Figure 1).

Table 1. The tulips cultivars at UASVM Cluj-Napoca

No.	Cultivars	Groups	Flower colour
1	'Yokohama'	I – Single early tulips	Yellow
2	'Veronique Sanson'	III – Triumph tulip	Orange with red
3	'Tender Whisper'	III – Triumph tulip	Fuchsia with white
4	'Paul Scherer'	III – Triumph tulip	Dark purple
5	'Salmon Impression'	IV – Darwin hybrid tulips	Light pink salmon
6	'Davenport'	VII – Fringed tulips	Red with yellow
7	'Fancy Frills'	VII – Fringed tulips	Pink
8	'Blue Parrot'	X – Parrot tulips	Violet
9	'Red Riding Hood'	XIV – Greigii tulips	Red

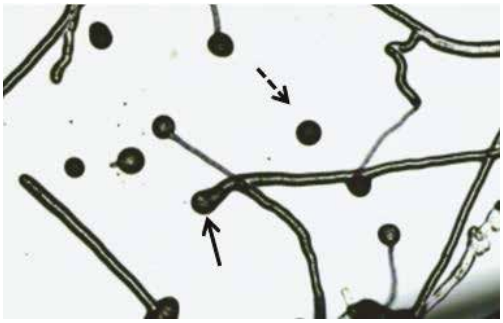

Figure 1. Pollen grain germination in tulips - magnification 10x (filled arrow - germinated grains; dashed arrow - non-germinated grains) (source:original)

Viability analysis

Viability of pollen grains was examined by means of the potassium iodide colouring test. In the presence of the colouring agent fertile grains with viable cytoplasm and spermatia will colour in black/dark brown while the sterile ones will remain colourless (Figure 2).

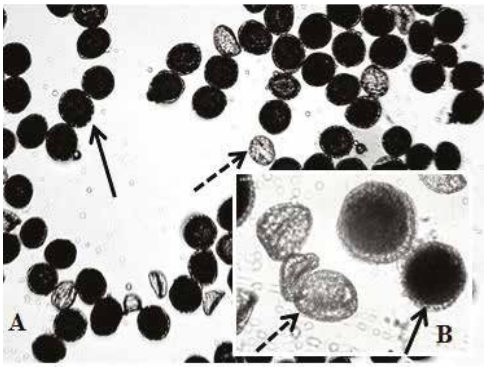

Figure 2. Pollen grain viability in tulips: A - magnification 10x; B - magnification 40x (filled arrow - viable grains; dashed arrow - non-viable grains) (source:original)

In this purpose, anthers were sunken immediately after collecting in the Carnoy fixative solution for a period of 1.5 hours then passed into alcohol until determination.

For high accuracy, in both determinations there were analysed about 300 pollen grains in several microscopic fields, then the rate (%) of germination and viability was calculated.

RESULTS AND DISCUSSIONS

In plant breeding the artificial cross pollination is a very important proceeding in creation of genetic variability, which will then be exploited by selection in order to obtain new cultivars with higher ornamental value than those on the market.

In tulip species there are multiple incompatibility barriers in interspecific hybridization as described by Kho and Baër (1971). To achieve a successful artificial hybridization, pollen grains should have a minimum 30% germination rate, very important to be taken in consideration when choosing the genitors in plant breeding programs.

The analysis of pollen grains of each cultivar of our tulip collection is presented in Figure 3.

Examining the results obtained there can be noticed that the highest germination capacity rate has been registered in 'Blue Parrot' cultivar (81.1%) while the lowest in 'Veronique Sanson' (7.8%).

It is also noticeable that only four cultivars ('Blue Parrot' - 81.1%, 'Davenport' - 78.3%, 'Red Riding Hood' - 35.9%, 'Salmon Impression' - 27.7%) have exceeded the 30% germination rate, the minimum level for a successful artificial hybridization.

Within the groups germination capacity varies as it follows: all the three cultivars belonging to group III - Triumph tulip, presented very low germination rate ('Veronique Sanson' - 7.8%; 'Tender Whisper' - 14.3% and 'Paul Scherer' - 11.0%) while in group VII - Fringed tulips - results are different. 'Devenport' records a rather high germination rate (78.3%) while in 'Fancy Frills' only 23.3% of the pollen grains germinated. This denotes that in cultivars of the same group pollen grains do not necessarily germinate in the same rate.

As far as viability is concerned data presented in Figure 3 reveal a higher rate than germination. Almost all cultivars, regardless the group they belong to, present a rather high viability rate between 81.7% ('Yokohama') and 96.1% ('Blue Parrot').

Cultivars of group III - Triumph, present a high viability level ('Veronique Sanson' - 93.9%; 'Tender Whisper' - 90.7% and 'Paul Scherer' - 86.0%) as compared to their germination rate which does not succeed to reach the necessary minimum 30%. The same situation is observed in 'Yokohama' and 'Fancy Frills' cultivars.

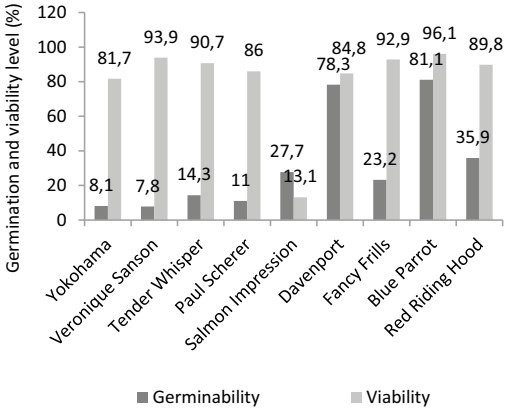

Figure 3. Pollen grain germination and viability in nine tulip cultivars, UASVM Cluj-Napoca, 2017

A special behaviour presents the 'Salmon Impression' cultivar (IV - Darwin hybrid tulip group) which encountered a rather low rate both in germination and viability.

According to the data presented in Figure 3 it can be noticed that the viability of pollen grains in all tested cultivars reached much higher levels than their germination rate, except the 'Salmon Impression'.

CONCLUSIONS

Based on these results there has been reached the conclusion that displaying a higher viability than germination rate might be due to the length of the drying and preservation period of pollen between collecting and analysis (4-5 days). This suggests that the fertile period of tulip pollen is rather short, only a few days.

As a consequence of the pollen viability and germination analysis in our tulip collection there can be concluded that only three cultivars ('Davenport', 'Blue Parrot' and 'Red Riding Hood') presented fairly high rates in both determinations, while the 'Red Riding Hood' cultivar slightly passed the required germination level. These results offer important information for selecting the best female/male genitor in tulip breeding programs for higher ornamental value.

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