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CYTO-PALYNOLOGICAL OBSERVATIONS ON SOME PEA (*Pisum sativum*) GENOTYPES

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Abstract

The biological function of pollen grains in plants is to transfer the genetic material from the male to the female reproductive organ. Pollen viability has a particular importance because it allows knowing the value of a genotype as a pollinator in various interfertile combinations or artificial hybridization. Pea (Pisum sativum) has an impressive nutritional profile and is considered to be an essential food for the proper functioning of the human body, especially since it is an important source of protein. The purpose of this study was to evaluate the viability of pollen grains to some pea genotypes and the percentage of pollen germination on artificial substrate (in distilled water). The anthers were harvested in the advanced flower bud phase from four pea genotypes experimented at SCDA Caracal, University of Craiova. The results obtained showed a high viability of pollen grains in all four peas genotypes tested, with values between 95.36-98.55%. On the other hand, it was found that on the artificial medium, after 24 hours the germination percentage was reduced (39.25%), only a small part of the pollen tubes presenting the entire content expelled. This suggests that the rainfalls during peas flowering can negatively affect the germination of pollen grains, by diluting the stigmatic liquid. Regarding the length of the pollen tubes after 24 hours, it was found that, on the artificial medium, the values recorded were higher than on the stigma, the elongation rate having a more accentuated rate at the beginning of germination, after which there is a gradual decrease of this rate.

Key words: *pea, cyto-palynology, pollen grains, viability, germination.*

INTRODUCTION

Pollen is a mass of fine, generally yellowish powder, made up of numerous grains and located on the surface of the anthers. At plants, pollen is a male product of the reproductive organs, having the role of fertilizing the floral ovules, in order to transform them into fruits or seeds (Dahlhausen et al., 2020).

The pollen grain is a microspore, representing the male gametophyte, usually reduced to two unclosed cells, each with a haploid male nucleus (n): the generative nucleus and the vegetative nucleus. A pollen grain has microscopic dimensions (15-250 microns), and can have different shapes. The pollen grain consists of the exine (outer membrane, with characteristic relief for each species); intina (inner membrane, cellulosic, translucent); faviol (the cytoplasmic viscous mass in which the two nuclei float); generative nucleus (from which male gametes are formed); vegetative nucleus (which controls the activity of the whole grain) and pores. Through the pores on the surface of the exine are made the exchanges with the external environment and also here, the pollen tube will come out from inside the grain.

The formation of fruit at different species is influenced by genetic characteristics (including pollen and pollinators) and environmental conditions (Vuletin Selak et al., 2014; Bonciu, 2019; Paraschivu et al., 2019). The reproductive system of a plant can also be a key to its survival and to maintaining the genetic diversity of populations (Russo Godoy et al., 2018; Bonciu et al., 2021).

A systemic understanding of how agriculture, the economy and environmental health are interconnected is essential for identifying best practices available (Paraschivu et al., 2020; Durău et al., 2021; Paraschivu et al., 2021). This is all the more necessary after some periods with serious consequences in the economy, like pandemic crisis which has put a lot of pressure on agriculture in a number of ways (Paraschivu and Cotuna, 2021).

Pollen morphology could be useful as an identity element for clarifying the classification of many plant species (Sharafi, 2011; Khaleghi et al., 2019). Pollen preservation is a common practice in breeding work, especially when there is no agreement between the date of flowering on female and male inflorescences (Dinato et al., 2020). Mature pollen, able to withstand a high degree of dehydration, can be easily preserved at low temperatures without the prior cryoprotection (Kulus, 2019).

Pea (*Pisum sativum*) is an annual herbaceous legume from the *Fabaceae* family, cultivated for its edible seeds and pods. The root system is strong and penetrates into the soil up to 40-60 cm deep, but, if the soil allows, it can penetrate up to one meter. The stem is hollow inside and slightly branched. The leaves are pinnately compound, alternately arranged, consisting of 2-3 pairs of leaflets, the last leaflet being transformed into a prison. Flowering takes place 30-50 days after sowing, and on a plant, flowering lasts 10-25 days. The fruit is a pod, straight or arched with a length between 3 and 12 cm. Pea seeds are yellow or green in the dry state.

The flowers, white or slightly purple-reddish, are arranged in the underside of the leaves, one for the early varieties and 2-5 for the late ones. The corolla has a butterfly shape. The stamens - the male reproductive organs - in peas are 9 in number through their filaments and the 10th free. In the middle of the flower is the gynoecium which is monocarpic - the carpels are modified leaves and adapted to the function of multiplication.

Pea is unpretentious to vegetation factors, well-adapted to temperate climates. It is a drought-resistant plant, but requires higher amounts of water during flowering to grain formation.

Transfer of pollen grains from the anther to the stigma of same flower is known as autogamy or self-pollination. Autogamy is a type of self-pollination which is made by the union of two Greek words (*auto* and *gamy*). It means autogamy is the “self-union” in which female gamete ovule and male gamete pollen grain are united and fused to form zygote that comes from the same flower. Autogamy is the closest form of inbreeding and leads to homozygosity. Such species develop a homozygous balance and do not exhibit significant inbreeding depression.

Pea plants are naturally self-pollinating. In self-pollination, pollen grains from anthers on one plant are transferred to stigmas of flowers on the same plant. The pollination is done directly, the pollen reaching the stigmas of the same flower, but cross-pollination is not excluded, when the pollen passes from one flower to another, through wind, insects, water and under human action.

MATERIALS AND METHODS

The purpose of this study was to evaluate the viability of pollen grains to some pea genotypes and the percentage of pollen germination on artificial substrate (in distilled water).

The anthers were harvested in the advanced flower bud phase from four pea genotypes experimented at SCDA Caracal, University of Craiova, in the year 2021. The four pea varieties experimented were as follows: Omega, Tiara, Favorit and Lehel.

For this study, pollen was harvested from ripe pea flowers, for each genotype, in full bloom. The viability of the pollen was tested by responding to the staining with a 1% acetocarmin solution. The pollen germination was observed by being cultivated on an artificial substrate (distilled water), for 24 hours. Cyto-palynological observations were made at room temperature (22-24^{0}C).

The microscopic examination was performed under the LCD digital display microscope (Optika, Italy), using 40x and 100x objective. Five slides for each pea variety and 10 microscopic fields from each microscopic slides were analysed under microscope, so a total of 50 microscopic fields. From each microscopic field 10 pollen grains were examined (500 pollen grains in total for each genotype). In the chosen microscopic fields, on the one hand, there were examined the red coloured pollen grains, which have completely preserved their viability and on the other hand, there were those with partial viability, which coloured light red. Also, there were unviable, uncoloured pollen grains. The viability of the pollen is directly proportional with the reaction intensity of colouring with a acetocarmine solution.

Pollen viability was calculated as the number of viable pollen grains divided by the total number of pollen grains, expressed as a percentage.

The length of the pollen tubes was also determined using the ocular-micrometer in conventional units (micron-μ).

RESULTS AND DISCUSSIONS

In terms of climatic conditions, in the experimental year (2021) there were deviations of the monthly temperatures average compared to the multiannual average, especially in January, February and April (Table 1).
In April, May and June, the monthly temperatures averages were below the multiannual average.

Table 1. The evolution of the main climatic factors during the vegetation period of the pea genotypes tested at S.C.D.A. Caracal (2021)

Specification		I	II	III	IV	V	VI
T°C	Mmin	-12.1	-8.9	-7.3	-2.8	4.3	9.6
	Mmax	12.3	21.1	19.4	27.3	31.1	37.0
	Mavr	1.4	3.2	4.9	9.7	17.3	19.8
Pp mm	Mtot	98	29.6	92.4	32.6	55.6	102.8
	Mltavr	30.8	26.3	34.2	47.8	58.6	69.7
	Diff	+67.2	+3.3	+58.2	-15.2	-3.0	+33.1
H %	Avr	95.3	85.5	80.8	73.1	69.5	82.1
	Min	65.1	29.6	18.6	17.7	17.1	32.9

T = Temperature; Pp = Precipitations; H = Humidity; Mmin = Monthly minimum; Mmax = Monthly maximum; Mavr = Monthly average; Mtot = Monthly total, Mltavr = Multiannual average; Diff = Difference; Avr=Average; Min=Minimum

The flowering date for the tested pea genotypes is shown in Figure 1.

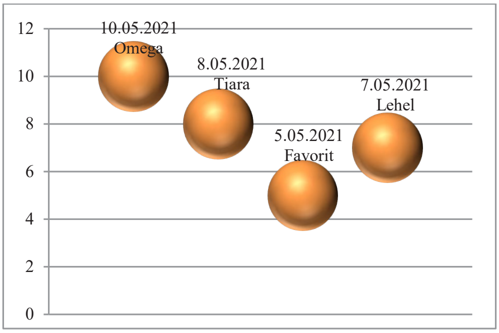

Figure 1. Date of flowering to some pea genotypes tested at S.C.D.A. Caracal (2021)

The pollen viability to pea genotypes tested at S.C.D.A. Caracal is showed in Table 2 and Figure 2. Thus, the highest value from this point of view was recorded by Omega genotype (98.55%), followed by Tiara (97.24%), Lehel (96.18%) and Favorit (95.36%).

Table 2. The pollen viability to pea genotypes tested at S.C.D.A. Caracal (2021)

Genotype	Npge	Nvpg	Nupg	Pollen viability (%)
Omega	500	493	7	98.55
Tiara	500	486	14	97.24
Favorit	500	477	23	95.36
Lehel	500	481	19	96.18

Npge=Number of pollen grains examined; Nvpg=Number of viable pollen grains; Nupg=Number of unviable pollen grains

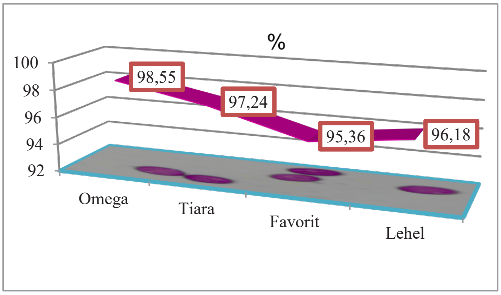

Figure 2. Graphical representation of pollen viability results in some pea genotypes

The results obtained showed a high viability of pollen grains in all four peas genotypes tested, However, as some authors suggest, even if pollen grains are viable, subsequent steps in pollination and fertilization may fail during heat. These heat sensitive steps are stigma receptivity of pollen, pollen grain retention on the stigma surfaces, successful pollen hydration, and pollen tube germination (Kaushal et al., 2016; Sita et al., 2017). Also, heat exposure negatively affected the pollen–pistil interaction at pea (Jiang et al., 2019).
Some climatic risk phenomena most oftenly limit the plant productivity (Ahmed et al. 1992; Hatfield and Prueger, 2015). Therefore, knowing the singular or cumulative effects of climate risks are the main criteria in the elaboration of a sustainable agricultural management (Paraschivu et al., 2015; Partal and Paraschivu, 2020).
Vital and nuclear colorations which indicate the presence of cytoplasm or enzymes are used to determine the viability of pollen (Nepi et al., 2005). Pollen germination can also be used as an indicator of viability.
The study of pollen grains has been the concern of many researchers since the last century. However, the interest for the pollen study is very evident from the works published in the last 10 years, works carried out with the most modern methods and which aim at the most

diverse aspects of cyto-palinology (Sita et al., 2017; Khaleghi et al., 2019; Dinato et al., 2020; etc.).

The generative growth of the pea plants and the increase of the biomass accumulation can be ensured by stimulating the fertility of the flowers, by increasing the percentage of fertile pollen grains and by the lengthening of the pollen tubes.

When several hundred pollen grains reach the stigma, each grain of pollen develops its own pollen tube in the stigmatic tissue. The fastest pollen tubes in terms of growth first reach the ovules and then fertilize them. Pollen grains with the fastest growing pollen tube carry genes that produce more vigorous offspring. Therefore, the growth rate of pollen tubes can establish a selective mechanism during early flowering (Figures 3,4).

Figure 3. Field images from SCDA Caracal (2021)

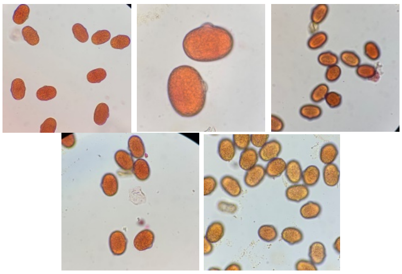

Figure 4. Microscopic aspects of cyto-palinologic studies at tested pea's genotypes

In terms of pollen germination and the length of the pollen tubes (Table 3), it was found that on the artificial medium, after 24 hours, the germination percentage was reduced (average 39.25%), only a small part of the pollen tubes presenting the entire content expelled. This suggests that the rainfalls during peas' flowering can negatively affect the germination of pollen grains, by diluting the stigmatic liquid. Regarding the length of the pollen tubes after 24 hours, it was found that, on artificial medium, the average values recorded were higher than on the stigma (842.72 µ versus 549.07 µ), the elongation rate having a more accentuated rate at the beginning of germination, after which there is a gradual decrease of this rate.

Table 3. The germination percentage and the length of the pollen tubes on the artificial growth medium versus stigma (average values after 24 hours)

Genotype	Pollen germination (%)	Length of the pollen tubes on the stigma (µ)	Length of the pollen tubes on the distilled water (µ)
Omega	41.2	618.3	985.4
Tiara	39.3	584.5	821.8
Favorit	37.6	473.4	693.2
Lehel	38.9	520.1	870.5
Average	39,25	549.07	842.72

CONCLUSIONS

Peas are in the group of foods known as legumes. Legumes are plants that produce pods with seeds or grains inside. Peas from harvested varieties at green maturity are used in human nutrition. At this stage, the beans are used fresh, in the form of pods (before seed formation), green/dried, frozen or canned grains and are usually combined with other foods.

The results obtained showed a high viability of pollen grains in all four genotypes of peas tested. Thus, the highest value from this point of view was recorded by the Omega genotype, followed by Tiara, Lehel and Favorit. The viability of the pollen was directly proportional with the reaction intensity of colouring with acetocarmine solution.

The pollen germination percentage on the artificial medium was reduced and only a small part of the pollen tubes showed the entire content expelled. This result suggests that the rainfalls during peas' flowering can negatively affect the germination of pollen grains, by diluting the stigmatic fluid.

The length of the pea pollen tubes was higher on the artificial medium (distilled water). From this point of view, the elongation rate having a more accentuated rate at the beginning of germination, after which there is a gradual decrease rate. However, the shelf life of pollen grains to pea tested genotypes is usually longer than in the artificial medium.

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