Journal of Applied Botany and Food Quality 82, 76 - 82 (2008)

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The correlation between ovule quality parameters and the seed yield at *Cyclamen persicum* MILL.

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(Received February 15, 2008)

Summary

Are there indicators that the seed yield at *Cyclamen persicum* is predetermined by the quality of ovules? This was the main question of these investigations.

The aim of our study was to investigate why only some of the available ovules develop into mature seeds. We surmised that the quality of the ovules played an important role in this. In order to corroborate this theory, we examined specific ovule parameters and their correlation with seed yield.

The parameters included the levels of callose in the nucellus, the heterogeneity of embryo sacs, the deviants in callose inclusion and the number of ovules examined by light and fluorescence microscopy.

There is still considerable disagreement on the biological significance of the inclusion of callose in ovules. In our study, we were able to show that the inclusion of callose is essential for fertilization in the case of *C. persicum*. This appears to contradict the findings reported for other plant species, where the inclusion of callose has been evaluated as a sign of ovule degeneration. However, the results of our study clearly demonstrate that seed yield is already determined by the maternal plant during the ovule development phase, i. e. shortly before and at the beginning of anthesis.

Some ovule parameters allow predictions to be made about the expected seed yield for the studied genotypes of *C. persicum*.

Introduction

Cyclamens are reproduced exclusively using seeds. Also, *Cyclamens* still have a high economic value, with approximately 25 to 30 million plants sold in Germany alone. In Germany, they represent about 10% of the total annual production of greenhouse flowering potted plants (ANONYMOUS, 2000). The number of cultivators and growers must steadily grow to meet the demand for potted plants. Since the trade in cyclamens is such big business, growers always strive to generate high quantities of seeds, independent of the genotype applied. However, differences appear in practice between individual genotypes and growing seasons. The causes for this are not fully known. In addition, a certain quantity of seed is commercially expected to produce a certain (stable) number of descendants. This requires a high quality of the seeds in respect of their germinating capacity and motivation force. We know from other plant families and types that not all ovules develop into ripe seeds (RODRIGO and HERRERO, 1997; AKHALKATSI et al., 1999). This phenomenon is thought to be an expression of an evolutionary, essentially sexual selection. It appears self-evident that, if the mother plant is deprived of nutrient resources, only a part of the ovules can thrive. Therefore, a competitive situation develops among the ovules. The ovules experience this competition repeatedly in a similar manner. Above all, before fertilization, the biochemical composition of the cytoplasm must be in the ovule, as well as in the embryo sac, for an optimal penetration of the pistil. This is obtained by a complex interplay of proteins, carbohydrates and inorganic substances (RAGHAVAN, 2000). Following fertilization, the ovules depend on nutrition from the mother plant. It is often reported that the competitive pressures occur at this stage. In *Quercus suber* L., only one of the six ovules (on rare occasions two) will generally fertilize (BOAVIDA et al., 1998) and develop into a ripe seed, blocking the development of the others. Even if two ovules are fertilized, only one continues to develop, while the other degenerates. Furthermore, it is reported that with *Cerasus vulgaris* MILL., a low yield of seeds correlates, among other things, with a high number of degenerated ovules, or anomalously developed embryo sac tissue (DYS, 1984). Experiments with buckwheat (*Fagopyrum* sp.), have shown that fertilization does not take place with abnormal embryo sac tissue (OBENDORF et al., 1992). With *Hormathophylla spinosa* (Cruciferae), only 50% of the existing ovules usually develop into ripe seeds (GOMEZ and ZAMORA, 2003). In this context, the phenomenon of callose inclusion in the ovules is highly interesting. It was not known whether the inclusion of callose in the nucellus of the ovules had a verifiably negative effect on pollen tube growth or pollination. To determine this, the studies for callose inclusion in the ovule were repeated for some flowers in which pollination was induced a few days before the start of anthesis. Examinations of the ovules of *Prunus armeniaca* showed that there was a great variance regarding development conditions (ALBURQUERQUE et al., 2002). Here, a genotype-dependent development of the embryo sac tissue was found at the time of the anthesis. Also with *Butomus umbellatus*, fertilization failure was linked to deformed or incompletely developed ovules (FERNANDO and CASS, 1997). Examinations at the Leibniz Institute of Vegetable & Ornamental Crops in Erfurt (Germany) have shown that higher yields were achieved with *Cyclamen*, if repeated dustings took place at the plants (EWALD and SCHWENKEL, 1997). This is conceivably to be seen as an indication of different development steps of the ovules of a placenta at the beginning of anthesis. Also, with *Pistacia vera*, it is reported that the ovules are still unripe at the time of the anthesis, and only if the pistil reaches the ovule, a ripe embryo sac becomes available (MARTINEZ-PALLE and HERRERO, 1998). In order to recognize causes for maturity, yield, and quality differences in the seeds, it is necessary to study the reproduction structures and processes prior to fertilization and in the early phases of the seed development.

Materials and Methods

Plant material

The plants were taken from the *C. persicum* cultivar 'Sylvia' (2n = 2x = 48). Ten plants had been cloned from a single plant (SK), while 20 more were obtained by sowing self-generated descendants of the same plant (SS). Furthermore, 20 plants from a population (SP), and ten plants of a autotetraploid clone (TK) with 96 chromosomes were used. The clones of the diploid kind 'Sylvia', as well as the autotetraploid clone, had been generated with a somatic embryogenesis procedure (SCHWENKEL and WINKELMANN, 1998). The process of cultivating these plants took place under relative constant temperature and humidity conditions in the greenhouse.

Light microscopic preparation

The ovaries were prepared from flowers at the beginning of anthesis and boiled in an aniline blue solution in a water bath at 100°C for 5 min. Then the ovaries were carefully crushed in propantriol on a slide. This method of preparation was carried out using dark field microscopy (Leitz DMRB, Germany) for the subsequent callose rating under UV light stimulation as well as for examining embryo sac heterogeneity. Four different examination periods were selected for the callose rating (three days before the start of anthesis, the anthesis itself, three to five days after the start of anthesis, and ten days after the start of anthesis).

To identify the deviants from the callose synthesis, all specimens that had been rated according to the extent of the callose inclusion were examined. For the embryo sac heterogeneity studies, ten plants were used from each genotype. Five flowers were used from each plant so that a total of 50 flowers were included in the study for each genotype. At least 100 ovules from each flower or placenta were analysed for their embryo sac surface areas, which gave a total volume of 5000 ovules per genotype for this study. In total, approximately 20.000 ovules were analysed. The surface area measurement of the embryo sac was carried out according to the planimetric method using the BioSciTec CellExplorer2001.

Statistical analyses of embryo sac heterogeneity

For the purposes of analyzing the parameter „embryo sac heterogeneity", the coefficients of variation (as percentages) for all flowers and ovaries were calculated from the quotient of standard deviation and mean. The mean values for the resultant coefficients of variation were then determined, and a Box & Whisker plot was prepared for each plant of each genotype. In order to allow a comparison between the individual genotypes, the overall means of the coefficients of variation for each genotype were calculated.

Statistical analyses of callose inclusion

Four different procedures were employed for the evaluation of callose inclusion in the ovules. A score of 0 means that there was no callose inclusion in the ovules, a score of 1 that there was the beginning of callose inclusion with some callose in the ovules, a score of 2 that two thirds of the ovule area were filled with callose and a score of 3 that the ovule was completely filled with callose.

The plant appraisal scores (rating method) were ranked and then subjected to a Mann-Whitney rank sum test. In order to evaluate the extent of callose inclusion in ovules during the various phases of ovule development, the quotient of the rank sums and number of investigated ovules per genotype was calculated and plotted in a line graph.

Statistical analyses of seed yield

For the statistical analysis, we used STATISTICA 6.1 (StatSoft GmbH Germany) and Microsoft Office Excel 2003. To analyse the seed yield, the number of seeds per seed vessel was recorded. This gave the total seed yield. This includes the seed yield for each plant, taking into account biological selection and the competition between the ovules as well as the vitality (and therefore the quality) of the mother plant. The total seed yield is composed of the net yield, the gross yield and the seed vessel yield. The net yield indicates the number of yielded seeds per seed vessel. The gross yield describes the number of seeds in an ovarium that have been developed from the available ovules per placenta. Finally, the seed vessel yield describes the proportion of yielded vessels in relation to the number of pollinated flowers. To allow a comparison between the different genotypes, the variation co-efficient of all flowers were calculated, following which we determined the mean values of the variation co-efficients of all genotypes.

Correlation analyses

Regression analyses were used in order to determine the correlation between embryo sac heterogeneity and the extent of callose inclusionas well as the deviations from standard (rating appraisal) callose inclusion in the ovules.

ANOVA (analysis of variance) and MANOVA, with a subsequent Newman-Keuls test, were used for the statistical analysis of the surface area of embryo sacs.

In order to test for correlations between ovule quality and subsequent seed yield parameters, regression analyses and Spearman's rank correlation tests were used.

Results

Callose inclusion

A phenomenon that was observed in the not pollinated flowers of *C. persicum* was the temporal occurrence of callose inclusions in the cell walls of the nucellus. A particularly large amount of callose was included at the start of anthesis. It was found that the intensity of the inclusion increased or decreased at different points during the flower development (Fig. 1). The callose inclusion began three days before the start of anthesis, during which a high concentration of callose was seen in the area of the chalaza (Fig. 2A). However, in the remaining areas of the nucellus, only a small concentration of callose was observed. This means that, although it was very easy to differentiate the nucellus from the remaining ovule after UV stimulation, there were no large deposits of callose in the cell wall area. At the beginning of anthesis, the concentration of callose greatly increased in the area of the nucellus, and all cell walls of the the embryo sacs were affected by callose inclusions from this point on (Fig. 2B). The SS was the one genotype whose values were lower than the others , three days before and 3-5 days after the start of anthesis (Fig. 1). This variation however

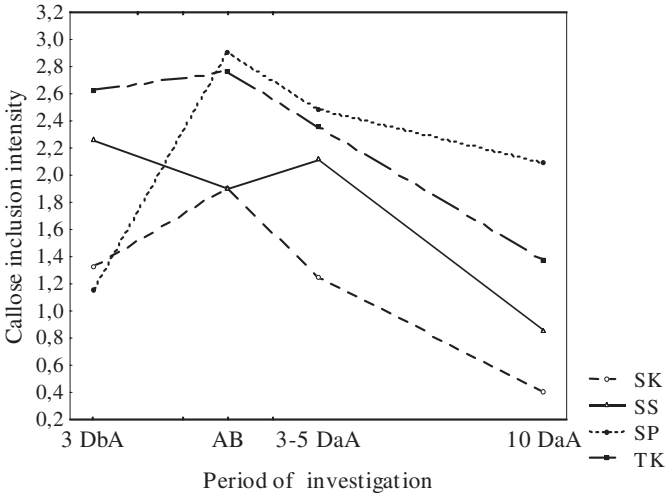

Fig. 1: The levels of callose inclusion intensity (all genotypes) at different times of flower development, multifactoral analyses of variance α = 0.05. 3DbA = three days before anthesis. AB = at the beginning of anthesis. 3-5 DaA = three to five days after anthesis. 10 DaA = ten days after anthesis. $n_{SK, SS, SP}$ = 120 flowers = 12000 ovules, n_{TK} = 86 flowers = 8600 ovules.
(0 - no callose inclusion in the ovules. score 1 - beginning of callose inclusion. score 2 - ovule was filled with callose to 2/3 of the ovule area. score 3 - ovule was completely filled with callose).

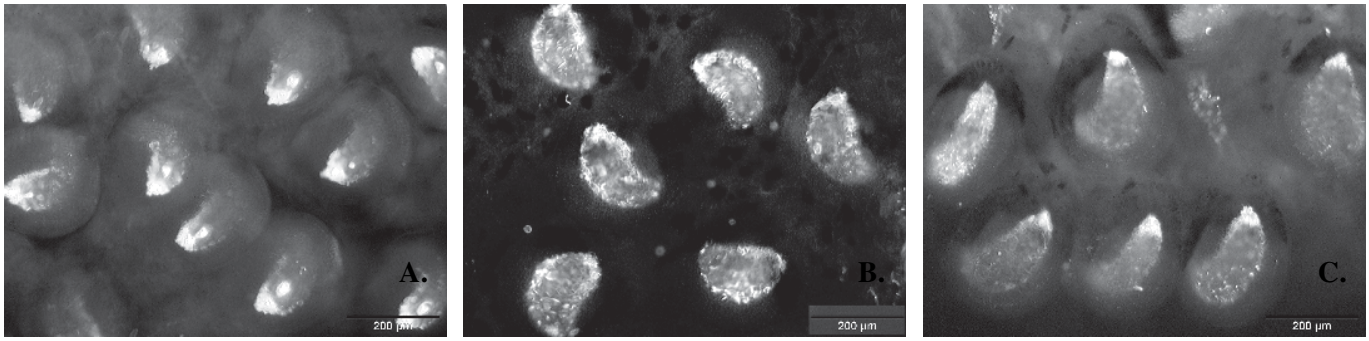

Fig. 2: Callose inclusion in the ovules at different times of flower development of genotype SP. A. three days before the beginning of anthesis. B. during the beginning of anthesis. C. five days after anthesis. The picture shows the area of the embryo sac inside the ovules with callose inclusion.

was not significant. During the examination of callose inclusion three to five days after the start of anthesis, the inclusion was observed to decrease gradually (Fig. 2C). The intensity of fluorescence decreased and it was now possible to identify areas in the nucellus that were free from callose inclusions. This was even more evident ten days after the start of anthesis, at which point the callose inclusion had almost completely disappeared in the area of the nucellus. Only a weak fluorescence could still be detected, which meant that it was no longer possible to differentiate clearly between the nucellus and the rest of the ovule.

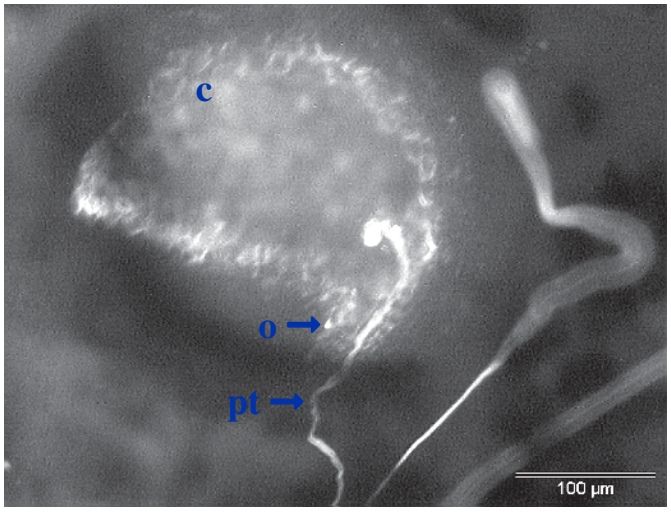

Fig. 3: Pollen tube penetration in the ovule through the obturator. Callose inclusion in the nucellus and the obturator (o = obturator, pt = pollen tube, c = callose inclusion in area of embryo sac).

The growth of the pollen tubes in ovules with callose inclusions in the area of the nucellus and the micropyle was not affected or inhibited in any way. The callose included in the area of the obturator or the micropyle clearly served as a type of guide for the orientation of the pollen tubes (Fig. 3). It was observed several times that the pollen tube grew in this precise area of the ovule. In each case, only one pollen tube grew in an ovule. Furthermore, seed development was established in all genotypes after pollination.

Callose inclusion deviants

It was observed in all genotypes that some ovules from the same ovary had homogeneous levels with regard to the extent of the callose inclusion, while others did not. This had different characteristics and will be termed deviants (Tab. 1). The deviants were characterised by the fact that the intensity of the fluorescence was weaker or noticeable stronger. In a period between three days before and three to five days after the start of anthesis, the genotype SK had the most deviants with 15.0%, while genotypes SP (2.2%) and TK (1.9%) had the fewest. This showed that in addition to the previously recorded structural irregularities, there were also physiological irregularities in unpollinated ovules.

Embryo sac heterogeneity

At the start of anthesis, there was a high heterogeneity regarding size and form of the embryo sac within the ovules of an ovary (Fig. 4). These differences, however, were also observed between different flowers on a plant, as well as between individual plants of a genotype. The comparative studies of the individual embryo sacs were based on the different embryo sac surface areas. In order to compare the genotypes with each other, the coefficients of variation (as percentages)

Tab. 1: Callose deviants at different times. 3 DbA = three days before the beginning of anthesis. AB = at the beginning of anthesis. 3-5 DaA = three to five days after beginning of anthesis. 10 DaA = ten days after the beginning of anthesis.

	Period for examination											
	3 DbA			AB			3-5 DaA			10 DaA		
Genotype	total invest. ovules	deviants	%	total invest. ovules	deviants	%	total invest. ovules	deviants	%	total invest. ovules	deviants	%
SK	1501	102	***6.8***	1929	57	***2.9***	1399	210	***15.0***	1953	52	***2.7***
SS	2340	22	***0.9***	2246	40	***1.8***	2135	141	***6.6***	1915	155	***8.1***
SP	2778	26	***0.9***	4453	58	***1.3***	3348	75	***2.2***	2955	43	***1.5***
TK	2496	18	***0.7***	2496	10	***0.4***	1548	29	***1.9***	373	10	***2.7***

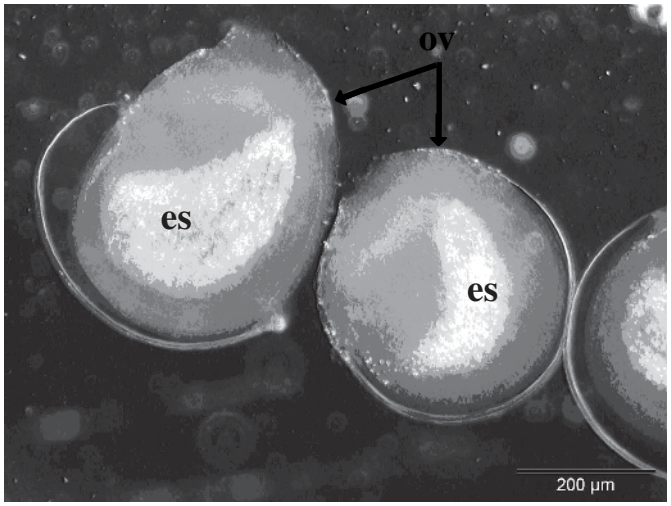

Fig. 4: Heterogeneity in the size of ovules and of embryo sacs in ovules at the beginning of anthesis (ov = ovules; es = embryo sacs)

of all flowers were calculated. It was observed that the genotype SK (cv% = 18.0) had the largest variability of all individual plants (Tab. 2). There were significant differences between the genotypes. The total values of the individual plants fell into a wide range, similar to the values previously established within a placenta. With the SS genotype, there was also a wide range of values, although the range was narrower than with SK. SP, on the other hand, displayed the least variation (Tab. 3). The values were basically characterised by their relative homogeneity. TK was also found to be relatively homogeneous with regard to these values. As a result, it was possible to identify differences regarding the embryo sac surface area on a placenta, both between different ovaries of an individual plant and between different individual plants of a genotype. In addition, the examined genotypes varied considerably in terms of the range of values.

Tab. 2: Embryo sac area heterogeneity (see also picture 4) of all genotypes at the beginning of anthesis. Coefficient of variation cv in %, mean values with the same letter within the parameter don't vary significantly (Newman-Keuls Test, $\alpha = 0.05$).

Genotype	CV%
SK	18.0 **(a)**
SS	16.8 **(a)**
SP	13.1 **(b)**
TK	14.2 **(c)**

Tab. 3: Embryo sac surface area of all genotypes and normal range of the ovules concerning embryo sac surface area at the beginning of anthesis (scale 1:64 means 1 μm^2 = 64 μm^2 = area x 64).

Genotype	Normal range (area µm², 1:64)	Total number of ovules	Ovules at normal range (%)	Ovules out of normal range (%)
SK	361.9 - 461.9	6559	**46**	**54**
SS	287.0 - 387.0	5126	**45**	**55**
SP	246.2 - 346.2	8280	**68**	**32**
TK	376.2 - 476.2	5116	**47**	**53**

Relationship between the ovule parameters

It was possible to determine a relationship between the values of the embryo sac heterogeneity and the total mean values of the deviants from callose rating. Both factors correlated positively with each other. This correlation is highly significant for $p \leq 0.01$ (Tab. 5). These differences between plants could also be observed in the direct comparison of the individual genotypes. There was also a correlation between the heterogeneity of the embryo sacs and the extent of the callose inclusion. The two factors correlated negatively with each other. It was evident that a high embryo sac heterogeneity correlated with a weak callose inclusion in the embryo sacs. The homogeneity of the embryo sac surface area, however, correlated with a high callose inclusion. The same relationship between individual plants could also be observed between the genotypes under examination.
The genotypes SK and SS had higher variation co-efficients with regard to the embryo sac heterogeneity than the genotypes SP and TK (Tab. 2). It was also evident that the genotypes SK and SS had the lowest levels of callose inclusion at the start of anthesis. The genotypes SP and TK, on the other hand, had significantly higher levels of callose (Fig. 1).
There were also highly significant correlations between the mean number of ovules per ovary, the callose inclusion and the embryo sac heterogeneity (Tab. 5).

Seed yield

Only about 20% of all the ovules in the diploid genotypes developed into visually ripe seeds (Tab. 4). There were no significant differences within the diploid genotypes in terms of the gross yield (Tab. 4). Clear and sometimes significant differences within the examined genotypes were observed in the net yield as well as the seed vessel yield. The SP genotype returned the highest values for both parameters under examination, and SK had the lowest values within the diploid genotypes. These differences were also observed in the total seed yield. The SP genotype had the highest total seed yield. In contrast, the total seed yield for the SK genotype was the lowest, falling short – by a significant margin – of the total seed yield for the SP and SS genotypes.

Relationship between ovule parameters and seed yield

In this context, it was possible to establish a correlation between the previously determined and examined ovule quality parameters and the parameters of the seed yield (Tab. 5). The strongest correlations were found between ovule and seed parameters at the diploid genotypes.
It was evident that there was a strong correlation between the extent of the callose inclusion, the net yield and the seed vessel yield, furthermore between embryo sac heterogeneity, callose deviants and all parameters of seed yield.
The strongest correlation was observed between the net yield (seeds per vessel) and the extent of the callose inclusion and the embryo sac heterogeneity.

Discussion

The levels of callose inclusion were similar for all genotypes under examination. An increase in the callose inclusion was observed in all genotypes three days before and during the start of anthesis. Three to five days after start of anthesis, there was a clear reduction in the callose inclusion. As before, there is still disagreement over the precise development of this inclusion, the biochemical details of the callose synthesis and the biological nature or consequences of this phenomenon. Callose has a comparable biochemical structure to cellulose.

Tab. 4: Key data from all genotypes concerning the mean number of ovules per ovary and yield parameters gross yield (seeds per ovary), net yield (mean number of seeds per vessel), seed vessel yield (developed vessels after pollination), mean values with the same letter within the parameter don't vary significantly (t-Test, $\alpha = 0.05$).

Genotype	Mean number of ovules per ovary	Gross yield %	Net yield n	Seed vessel yield %	Total seed yield %
SK	145 **(a)**	21.0 **(a)**	30.5 **(b)**	23 **(b)**	4.6 **(b)**
SS	190 **(b)**	21.2 **(a)**	40.3 **(b)**	54 **(bc)**	12.5 **(a)**
SP	237 **(c)**	23.6 **(a)**	56.0 **(a)**	75 **(a)**	17.9 **(a)**
TK	211 **(d)**	13.1 **(b)**	27.8 **(b)**	40 **(bc)**	5.4 **(b)**

Tab. 5: Correlations between callose inclusion intensity, callose deviants, embryo sac area heterogeneity, mean number of ovules per ovary and yield parameters of diploid genotypes (SK, SS, SP). Regression analyses, with * marked values are significant $p \leq 0.05$ and with ** marked values are significant $p \leq 0.01$.

	Callose inclusion intensity	Callose deviants	Embyo sac area heterogeneity
Callose inclusion intensity	1		
Callose deviants	- 0.45*	1	
Embryo sac area heterogeneity	- 0.56*	0.76**	1
Ovules per ovary	0.75**	- 0.35	- 0.60**
Gross yield (seeds per ovary)	0.34	- 0.55*	- 0.54*
Net yield (seeds per vessel)	0.72**	- 0.57*	- 0.74**
Seed vessel yield	0.63**	- 0.49*	- 0.51*

It is a polyglucan of β 1→3 glycosidic bonded glucose elements that have predominantly linear bonds. Alongside this, 1→4 or 1→6 bonds can occur between neighbouring molecule chains caused by the build-up of hydrogen bonds. The polymer has a helical structure to which the aniline blue bonds. Under UV stimulation, this leads to a green-yellow fluorescence. The biosynthesis of the callose occurs on rosette-shaped protein complexes of the plasma membrane. It is assumed that the callose-synthase-complex and the cellulose-synthase-complex are identical. The enzyme responsible for this reaction is the cellulose-glycosyltransferase and is part of a membrane complex in the plasmalemma (STRASBURGER, 1998; RICHTER, 1988). In a normal situation, the cellulose synthesis as a main element of the cell wall is catalysed on this enzyme complex. Sometimes this reaction fails. Instead of β-1→4 glucan bonds, β-1→3 bonds are now linked. For this reaction, calcium is essential as a co-factor (GAYLE and FRANCESCHI, 2000). Both the cellulose-synthase-complex and the callose-synthase-complex can specifically or randomly react to biotic and abiotic stress (VERMA and HONG, 2001). It was demonstrated on *Gossypium hirsutum* that carbon can be included both in cellulose and in callose, benefiting from the presence of calcium (AMOR et al., 1995). Although callose inclusion has been observed in many studies about different plant families and representatives of plant families, it is still unclear whether this is a defensive mechanism on the part of the ovule against self-pollination (LE ROUX et al., 1996) or an early indication of the ovule's increasing degeneration (STÖSSER and ANVARI, 1982; DITTMANN, 1998; DETZEL, 1998), or even an indication of fungal infection of the ovule (COHAN et al., 1990; JACOBSEN and BERRY, 2002). In this context, however, the discussion is about whether callose inclusion leads to an intermittent isolation of the embryo sac from the surrounding tissues, as a result of the associated restricted permeability between the cell walls and the impaired metabolite transport between the cells or the tissues. Also under discussion is whether this causes an unimpaired, autonomous development of the embryo sac, and whether it protects it against damaging exogenic influences (KAPIL and TIWARI, 1978; TIWARI, 1982; KAPIL and BHATNAGAR, 1981; BOUMAN, 1984; COLOMBO and ANGENENT, 1999). During the development of the megaspore mother cell, or more precisely, during mitotic cell division, the occurrence of callose around the dyads, triads, and tetrads was a frequently observed phenomenon (PORCEDDU et al., 1999; TUCKER, 2001). This callose inclusion around these cells probably causes the degeneration of the three megaspores (RODKIEWICZ, 1970; HUANG and RUSSEL, 1992). Fundamental studies into callose inclusion in the context of embryo genesis were carried out on *Arabidopsis thaliana* (WEBB and GUNNING, 1994).

Callose inclusion has often been observed in connection with a sterility of this ovule. This was also the case with *Medicago sativa* (ROSELLINI et al., 1998 and 2003; KOLYASNIKOVA, 1985), and other *Medicago* types (ORYOL et al., 1986). Ovules with callose inclusion are therefore deemed sterile, not functionable (KNOX et al., 1986) and non-viable (DUMAS and KNOX, 1983). However, indications of degeneration are often associated with callose inclusion, as shown in studies on *Prunus dulcis* (PIMIENTA and POLITO, 1982), *Prunus armeniaca* (RODRIGO and HERRERO, 1997) and other *Prunus* types (STÖSSER and ANVARI, 1982). In *Carica papaya*, callose was found in 90% of the aborted ovules, in these cases in the region of the funiculus (BAUTISTA-CALLES et al., 1999). This indicates the selective permeability of the callose-affected cell walls, and the consequently impaired metabolite transport into the ovule. In contrast to the possible functions of the callose inclusion mentioned above, however, the question whether the callose inclusion may be linked to the pollen tube growth, or more precisely, with the pollen tube direction, is still in the focus of discussion. An example for this dicussion is the case of *Pistacia vera*, in which callose appeared primarily in the region of the ponticulus, where the cells of the ponticulus form and discharge callose. After pollen tube penetration, the ovule separates from the remaining tissue because of the callose (MARTINEZ-PALLE and HERRERO, 1995). In *Ipomoea purpurea,* it was observed that the transmission tissue in the stigma, through which the pollen tube has to grow, had a higher concentration of callose (DIAZ-PONTONES, 2000). Even in *Galanthus nivalis*, in addition to polysaccharides, proteins, lipids, and calcium, callose was found in the micropylar channel and these substances had a direct influence on pollen tube growth. Part of the reason for this was that the proportion of the substances greatly decreased after pollination, and these substances did not occur in sterile ovules (CHUDZIK and SNIEZKO,

2003). The same phenomenon was observed in a study about *Paspalum* in which the callose served as a type of guide, used by the pollen tube to enter the inside of the ovule via the micropylar channel (CHAO, 1971). The successful development of the pollen tube in a callose-affected ovule was also observed in *Arabidopsis thaliana* (RAGHAVEN, 2000). This theory is further supported by the fact that in fundamental studies on callose inclusion in *Petunia hybrida*, a periodicity of the inclusion was seen, showing that the inclusion begins during pollination, and ends after pollination (ESSER, 1963). Our own studies on *Cyclamen persicum* confirm this theory. On the one hand, the same degree of periodicity was observed, and on the other hand, we found on several occasions that pollen tubes were developing into callose-affected ovules. The callose concentration was especially high in the area of the obturator and the micropylar channel at the moment of pollen tube penetration, which probably means that the callose should be viewed here as a functional pollen tube attractant. Therefore, the callose inclusion can be defined as a phenomenon in functional ovules. This also corresponds to the fact that there is a negative correlation between the parameters 'embryo sac heterogeneity' and 'extent of the callose inclusion'. The callose inclusion and the associated biological efficacy is therefore higher in ovules with rather homogeneously developed embryo sacs. The deviants identified in the callose rating are therefore regarded as non-functional or partially functional ovules. This view is also supported by the close correlation between the embryo sac heterogeneity and the deviants from the callose rating. In addition to the different basic forms of the ovules on a placenta, it was also possible to identify heterogeneous shapes and surface qualities of the embryo sacs. The phenomenon of disparity between embryo sacs has been reported in many studies. In *Medicago sativa*, the embryo sac heterogeneity is regarded as the cause for poor seed yield (ORYOL and KAZACHKOVSKAYA, 1991). Embryo sac heterogeneity was even reported for *Prunus* sp. as an indication for the lack of a functional ovule, linked with a poor yield (EATON and JAMONT, 1964). With *Ipomoaea nil*, there was a connection between aborted ovules and simultaneous embryo sac degeneration (MIYAJIMA et al., 2003). This was also observed with olives (RAPOPORT and RALLO, 1990). With *Petunia inflata*, a correlation was reported between embryo sac heterogeneity and failed pollination, which also caused a reduced seed yield (LEE et al., 1997). Basic studies on *Arabidopsis thaliana* showed conclusively that a normally developed gametophyte was necessary for direct pollen tube growth, whereas defective ovules with abnormally developed embryo sacs were not pollinated (HÜLSKAMP et al., 1995). This demonstrates that pollination is possible only when a completely formed embryo sac, including egg apparatus, is present (SHIMIZU and OKADA, 2000). The shape and size of the *Cyclamen* embryo sac is determined by the shape and size of the ovule. A small embryo sac indicates that the ovule has formed abnormally. Therefore, it can be interpreted as a failed formation with limited functionality. On the basis of the fore-going studies, the ovule characteristics described earlier – i.e. 'embryo sac heterogeneity', 'callose inclusion', and 'callose inclusion deviants' – were classified as ovule quality parameters.

It appeared that only a fraction of the large number of ovules available on a placenta developed into ripe seeds. This meant that in the diploid genotypes, only approx. 20% of all ovules developed into ripe seeds (only 13% of the TK genotype). The abort rate was therefore between 80% and 87%. This phenomenon is well documented: With *Melilotus officinalis*, only one ovule out of eight developed into a ripe seed (AKHALKATSI et al., 1999), with *Prunus armeniaca*, only one in two ovules succeeded (RODRIGO and HERRERO, 1997), the same success rate observed for *Quercus suber* (BOAVIDA et al., 1999), while, in *Hormatophylla spinosa*, the proportion of ripe seeds from the existing ovules usually falls short of the 50% mark (GOMEZ and ZAMORA, 2003). It is therefore assumed that the failure on the maternal side is determined (AKHALKATSI et al., 1999; KARKKAINEN et al., 1999), among other causes, by a lack of available nutrients (STEPHENSON, 1981). In addition, ovule sterility is also a major reason for poor seed yield, as was demonstrated in the case of *Trifolium repens* (THOMAS, 1996). However, a relationship has also been frequently established between abnormal ovule development, and therefore abnormally formed embryo sacs, and a poor seed yield (ORYOL and KAZACHKOVSKAYA, 1991; EATON and JAMONT, 1964). This kind of relationship was also found in the present studies. With an increasing homogeneity of the embryo sacs of the ovules on a placenta, the seed yield was also high, whereas a lower seed yield correlated with a high heterogeneity of the embryo sacs. This is possibly an indication of the vitality and therefore the quality of the mother plant. There was also a correlation between the extent of the callose inclusion and the seed yield. This could also be seen as an indication for the quality of the mother plant, much in the same way as the embryo sac heterogeneity. From this, it can be concluded that a high quality of the mother plant also means an increased availability of nutrients for the developing seeds and ovules. This also benefits the seed yield. The correlation of the deviants from the callose rating and the seed yield also supports this theory. Consequently, it may be concluded, on the one hand, that the ability of the mother plant to create a large number of ovaries is an indication for its vitality, and, on the other hand, that the quality of this mother plant allows a high seed yield to be achieved. The presence of any irregularities in the ovule or seed development can be measured and predicted reliably on the basis of the parameters 'embryo sac heterogeneity', 'callose inclusion in the ovules', and 'callose inclusion deviants'. As is shown in the present studies, however, the parameters 'number of ovules', 'embryo sac heterogeneity', 'callose inclusion in the ovules' and 'callose inclusion deviants' allow predictions to be made about the expected seed yield for the studied genotypes of *Cyclamen persicum*.

Our results demonstrated that seed yield is determined in early phases of seed development, i.e. at the ovule stage.

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