UDC 582.573.76:581.33

CORRELATION OF DEVELOPMENTAL STAGE AND ANTHERS SIZE OF SOME *HEMEROCALLIS* X *HYBRIDA* HORT. CULTIVARS

Tatiana Nikolayevna Kuzmina

Nikitsky Botanical Gardens – National Scientific Centre
298648, Republic of the Crimea, Yalta, urban vil.Nikita
tnkuzmina@rambler.ru

Introduction

According to current conception, anther of angiosperm plants is considered as an integral and self-regulatory system, which includes coordinated processes happening consecutively and condition its development and creation of male gametophyte [2, 11]. Correlation in genesis processes of anther`s wall and male gametophyte [12, 15] allows to suppose interconnection between growth and development of male generative structures of flowering plants. Studies in such a field are usually aimed at determination of maximum stage when anther is possible to introduce into culture in vitro to get haploid plants [1, 4, 8, 10, 14], what is high-usage in breeding [4, 6]. Though data of development periodization of male generative structures allowing for genesis of a flower and anther elements are also of great importance for cytological analysis of microsporogenesis and prognostication of pollen quality, what is necessary for sorting out the parent forms of selection material. One of the main ornamental cultures which is selected in Nikitsky Botanical Gardens is *Hemerocallis* x *hybrida* Hort. That is why it`s extremely important to investigate regularities peculiarities of the high quality material genesis. Purpose of this study is to determine interconnection between morphometric parameters of anthers and developmental stage of male generative structures of some *H.* x *hybrida* cultures with different type o ploidy.

Objects and research methods

Four cultivars of *Hemerocallis* x *hybrida* Hort. were used as objects of the investigation. They are cultivated in genefund collection of Hemerocallis hybrida in Nikitsky Botanical Gardens (supervisor of the collection is I.V. Ulanovskaya), where Pandora's Box and Wally Nance are diploid and Anna Warner and Cherry Eyed Pumpkin – tetraploid. Different sized buds of study cases (minimum was 0,1 sm) were used to determine morphometric parameters of anthers and developmental stages of male generative elements. Development stages of anther were determined on temporary preparations stained with 1% acetoorseine. Preparation analysis was carried out with microscopes "Jenaval" (Carl Zeiss) и AxioScope A.1 (Carl Zeiss) applying method of light field. Statistic data processing was conducted using a set of application programs Statistica 6.0. Reliability of differences between variants was assessed by Student`s t-criterion on 5% level of significance which guarantees 95% confidence probability.

Results and discussion

Genesis of microsporangium wall and pollen grain are thoroughly described before [7], within this research we emphasized just key stages of anther`s structure development. Morphometric data of anthers belonging to four cultivars of *H.* x *hybrida* are presented in the table below according to periodization of sporangium development and main stages of male

gametophyte formation. In accordance with agreed periodization of anther development three periods were marked out: premeiotic, meiotic and postmeiotic [5, 12]. During premeiotic period thanks to intensive mitotic division formation of microsporangium wall occurs and sporogenous tissue is founded for further microsporocytes development. Differentiation of cellular wall layers of anther takes place on meiotic stage, while microsporocytes start for meiotic division and as a result it conduces to development of tetrads with haploid microspores. Postmeiotic period begins with the decay of microspores tetrads and finishes by process of gametophytogenesis that is development of a pollen grain and anther ripening.

Study cultivars of *H.* x *hybrida* on the starting stages of microsporangium development are fixed in anthers at height of 0,1 sm. On this stage anthers of diploid and tetraploid cultivars don`t differ by height. Though reliable differences of anther morphologic parameters of diploid and tetraploid cultivars are typical in meiotic period (t=3,12).

Table

Anther morphometric characteristics of some *Hemerocallis* x *hybrida* cultivas during development of male gametophyte

Period of microsporangium development	Stages of anther and male gametophyte development	Anther`s height, sm			
		Diploid cultivars		Tetraploid cultivars	
		Pandora's Box	Wally Nance	Anna Warner	Cherry Eyed Pumpkin
Premeiotic	Microsporangium formation, foundation of sporogenous tissue	0,15±0,02 0,1–0,2	0,15±0,01 0,1–0,15	0,15±0,02 0,1–0,2	0,15±0,01 0,1–0,2
	Formed microsporangium wall, microsporocytes	0,3±0,04 0,25–0,4	0,31±0,05 0,20–0,35	0,35±0,04 0,25–0,5	0,33±0,03 0,2–0,45
Meiotic	Degeneration of the middle wall layer of microsporangium and tapetum; meiosis, formation of microspores tetrads	0,51±0,02 0,38–0,6	0,41±0,05 0,36–0,45	0,64±0,02 0,6–0,69	0,59±0,04 0,5–0,7
Postmeiotic	Formation of fibrous bulges in microspore endothecium, formation of sporoderm, differentiative mitosis	0,69±0,01 0,6–0,75	0,63±0,03 0,5–0,7	0,8±0,03 0,7–0,9	0,89±0,03 0,8–0,97
	Formed anther`s wall Bicellular pollen grains	0,83±0,01 0,79–0,9	0,79±0,01 0,78–0,8	1,00±0,02 0,9–1,1	1,1±0,02 1–1,2

Note: there are an arithmetic average and a standard mistake (m±x) above the line; under the line there are limits of a character variation (min -max).

Thus, meiosis and formation of microspores tetrads of such diploid cultivars as Pandora's Box and Wally Nance occurs when anthers are 0,4-0,5 sm by height, while for tetraploid cultivars - Anna Warner Cherry Eyed Pumpkin – this period happens to be when height of anthers reaches 0,5-0,7 sm.

It`s a well-known fact the optimal stage when anthers are possible to introduce in culture in vitro is a stage of microspores [3, 9, 14], when autonomy of microspores takes place and they start for realization of gamotophytogenesis determinate program, or under conditions of culture in vitro – sporophytogenesis [1, 12, 13, 15]. In this period anther height of diploid cultivars of *Hemerocallis* x *hybrida* ranges from 0,5 – 0,75 while tetraploid cultivars get 0,7-1 sm.

Reliable difference in anther height of diploid and tetraploid species was determined in postmeiotic period [t=9,24). Pollen grains of diploid cultivars get ripening when anther height

is 0,8 sm on average, tetraploid species - 1-1,2 sm. Thus *H. x hybrida* cultivars as ploidy degree increases anther morphometric parameters rise up during meiotic and postmeiotic periods; anthers of tetraploid species are much larger.

Analysis of anther`s height and stage of its formation define the anther`s growth and its development are conjugated, what helps to identify critic stages of its development, allowing for anther`s morphometric parameters. Applying the most available morphometric methods for assessment the stages of the male generative sphere development of *H. x hybrida* cultivars material optimizes process of material sorting out for cytologic analysis of microsporogenesis in order to reveal anomalous cases during pollen formation and while introducing the anthers into culture in vitro.

Conclusions

In terms of the study anther`s morphometric parameters of diploid (Pandora's Box, Wally Nance) and tetraploid (Anna Warner, Cherry Eyed Pumpkin) *H.* x *hybrida* cultivars were determined during the principal periods and stages of sporogenous tissue foundation and formation of male generative structures.

It was presented that anther`s height of study diploid cultivars in meiotic period makes 0,4-0,4 sm on average, while tetraploid – about 0,6 sm. Phase of microspores takes place when anther`s height reaches 0,6 sm and 0,8 sm, relatively for study diploid and tetraploid cultivars.

Reliable difference between anthers of diploid and tetraploid cultivars was revealed during meiotic and postmeiotic periods.

Findings allow to consider anther`s height of study *H.* x *hybrida* cutivars as an indirect indicator of anther development stage, necessary for visual assessment the material for cytologic analysis of mictosporogenesis of the species material, as well as for introduction the anthers into culture in vitro.

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The article was received at editors 17.11.2014.

Kuzmina T.N. Correlation of developmental stage and anthers size of some *Hemerocallis* x *hybrida* hort. cultivars // Bull. of the State Nikit. Botan. Gard. – 2015. – № 115. – P. 50-53.

Main genesis stages of microsporangium and elements of male generative sphere were determined for diploid (Pandora’s Box, Wally Nance) and tetraploid (Anna Warner, Cherry Eyed Pumpkin) cultivars of *Hemerocallis* x*hybrida* Hort. during anther`s development.

Key words: *anther; pollen-grain; microsporogenesis; microspore; ploidy; Hemerocallis x hybrida hort.*