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FEATURES OF PARENTS' SELECTION AND EFFICIENCY OF ARTIFICIAL HYBRIDIZATION OF *CANNA* L.

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Розглянуті можливості штучної гібридизації з використанням сортів з різних груп канни та перспективність залучення інтродукованих видів для генетично-селекційного збагачення сучасного сортового різноманіття канни.

***Метою роботи** був підбір батьківських пар за визначенням індексу фертильності пилку інтродукованих видів і сортів різних груп канни для отримання селекційного різноманіття форм стійких при вирощуванні в південному посушливому регіоні України (на прикладі м. Кривий Ріг).*

***Матеріали та методи**. Дослідження проводились на 7 інтродукованих видах і варіаціях (C. tuerckheimii Kraenzi.; C. indica var. warscewiczii Nob. Tanaka; C. iridiflora Ruiz&Pav.; C. flacida Salisd.; C. indica var. edulis Ker Gawl.; C. indica var. coccinea Mill.; C. indica L.) та 28 сортах канни (з них 67,8 % закордонної селекції). Цитологічний аналіз пилку проводили згідно прийнятої методики(за йодною реакцією крохмалю). Гібридизацію здійснювали з використанням існуючих розробок для канни. Строки гібридизації та час проведення штучного запилення були встановлені з урахуванням еколого-кліматичних умов промислового регіону.*

***Результати.** На основі цитологічного аналізу пилку інтродукованих видів і перспективних сортів канни підібрані батьківські пари для штучної гібридизації з метою генетично-селекційного збагачення її різноманіття та отримання зразків, придатних для вирощування в степовій зоні України, зокрема в екологічно-дестабілізованому регіоні. Розроблений підхід до відбору вихідних форм канни для штучної гібридизації на основі цитогенетичного аналізу пилку.*

***Висновки.** За показниками якості пилку (індекс фертильності) в період завершення бутонізації виділені перспективні інтродуценти канни для подальшого використання батьківськими формами в гібридизаційних схемах. На основі проведеної штучної гібридизації перспективних для степової зони і промислового регіону України інтродуцентів роду Canna визначені найбільш результативні комбінації схрещувань. При цьому при схрещуванні сортів групи Крозі між собою отримано найбільше результативних комбінацій. З отриманого фонду гібридних рослин канни виділені селекційні форми за комплексом декоративно-цінних ознак рослин і введені до колекційного фонду та подальшого їх впровадження в озеленення*

***Ключові слова:** види та сорти роду Canna, пилок, стерильність, індекс фертильності, гібридизація, батьківські форми, селекція*

1. Introduction

Genus *Canna* (*Canna* L.) unites about 50 species of permanent grass plants of the Cannaceae family order of the ginger family [1, 2]. The centre of origin of most species of this genus is tropical and subtropical areas of Central America, Islands of the Caribbean Basin, the northern equatorial Andes and the north Brazilian provinces. Now this culture is introduced in Asia, Africa, India and China [3, 4]. Since the 17th century, the garden *Canna* culture has taken a prominent place in the decorative landscaping of Europe [5, 6].

Very few varieties from the modern decorative forms of *Cannes* are used in landscape design. During the last decades a part of modern foreign varieties turned out to successfully be introduced in Ukraine, even in its steppe zone. The varied fund of the introduced plant of *Canna*, created in the Kryvyi Rih Botanical Garden of NAS of Ukraine (farther – KBG), is a confirmation of this [7]. It is the need to get new *Canna* varieties, adapted to different ecological and climatic conditions of the regions of Ukraine, became a prerequisite for this work.

There are 7 species and forms and 37 varieties of *Canna* of foreign and domestic breeding (foreign varieties make up 67 %) in this collection. Of these, 17.0 % of varieties of group I (*C. foliosa-parviflora* hort.), 57 % of varieties of group II (Crozy group) and 26 % – group III (*C. orchiodes* Bailey. or Italian group) [7, 8].

2. Literature review

The culture of *Canna* has taken an important place in ornamental plant beautification in Europe since 17^{th} century. The species or varieties of *Canna* were used in landscape design of the time. All modern polyploid species of *Canna*, in fact, are made on basis of 5 parents' species, which were involved in hybridization from 1848 to 1892. They are now united under name of *Canna hybrida hort. ex Backer*. So, for 45 years' period the fast transformation of the uncultivated plant into the orna-

mental plant has taken place mostly because of distant hybridization and poliploidy. The first stage took place in the middle of the XIX century, when the French plant breeder Theodore Anne created the first hybrids of *Cannes* from crossing species *C. indica* and *C. glauca.* These hybrids became known as *C. annaei* hort. [7]. The second stage of hybridization involves the work of Lyon plant breeder Pierre Antoine Crozier. He got the first large-flowered hybrids by crossing *C. indica* and *C. indica var. warszewiczii.* From further hybridization of these forms with *C. ehemannii* plants, this breeder obtained a garden form, which was later called "Canna Crozier" or "French Canna" [9]. This form has become the prototype and standard of the modern garden form "Cannes Crozier" [10]. Hybrids, called iris-like or orchid-shaped Italian Cannes (*C. orchioides* Bailey) are the result of the third stage of breeding cultivation of these plants. They were obtained by crossing Crozier's hybrids (as the maternal form) with different North American species, in particular, *C. flaccida* Salisb.

Nowadays there are some varieties (Queen of Italy), which were gotten as a result of vegetative mutation, but creation of better varieties was connected with repetitive cycles of hybridization and polyploidy [11, 12]. Te breeding potential of this plant is high because the genofond of *Canna* genus is little used [13]. Despite the variety of modern species of *Canna* (about 1000 according to some informational resources), only about 200 species are used in practice. In decorative floriculture of Ukraine species of foreign selections prevail.

T. Theophilova developed a classification of *Cannes* by flower shape. On this basis, all the representatives of the collection of *Canna* can be divided into three groups:

I – *Canna foliosa-parviflora* hort. – small-flowered species;

II – *C. Crozy* hort. – varieties with open gladiolus-like flowers;

III – *C. orchiodes*) – varieties with large flowers which resemble Cattleya's orchid [11].

However, these varieties of *Canna* are little adapted to the soil and climatic conditions of Ukraine. The leading center of introduction and breeding work for the creation of native varieties of *Canna* is located in the Crimea (Nikita). Weather and climatic conditions of this territory are more suitable for growing the plants of the Cannaceae family [14, 15]. Therefore, the selection of domestic varieties, most adapted to the new conditions of growing, is very important [16].

The Kryvyi Rih region is situated in the southern agroclimatic arid region of Ukraine. The sum of active air temperatures of atmospheric is from 3000 ° C in the north of the region to 3200 °C in the south [17, 18]. The territory of this region receives an average of 400–450 mm of precipitation per year. Two thirds of the rainfall account for the warm part of a year (300-320 mm). Atmospheric precipitation in the winter months falls less – 100–130 mm. During all summer months there is a deficit in the balance of humidity, although 60–70 % of annual rainfall falls during the growing season. This is due to high summer air temperatures and a significant predominance of evaporation over the sum of precipitation. The moisture deficit in June is – 76 mm, in July – 141 mm, in August – 142 mm. The total annual deficit of moisture is equal 350–420 mm [19].

The city of Kryvyi Rih is reported to have the high complex index of atmosphere pollution [20].

The complex of hard climatic and ecological conditions of the region has an impact on the existing variety of plants in landscape gardening and peculiarities of their growth and development [21]. The inventory of green areas of Kryvyi Rih showed that only 7 species and 12 varieties of the perennial herbage plants are used in greening the city. There are only 2 species of *Cannes* among them, 4 varieties of II group (Crozy group) and 2 varieties from III group (orchid-like group or Italian group). This confirms the importance of breeding work and introduction of promising taxa of this genus and their continued use in planting of greenery of the region [22, 23].

According to the literature, artificial hybridization, using introduced varieties and species of *Canna,* is rarely successful [24]. A different number of chromosomes in cultural forms and species plays a large role [25]. Matveeva (1980) found out that the ideal valence for *Canna* is likely to be a triploidy which allows valuable decorative characters to appear.

Triploid forms were widely used in artificial hybridization. Tetraploids received spontaneously became useful only as an interim stage for creation of more effective triploids [9].

It is well known, that the quality control of pollen of species and varieties determines the possibility of using them in breeding work [26, 27]. In a range of works certain records about comparative study of pollen of some species genes and varieties of *Canna* L. on the basis of morphological characters are given [28, 29]. An average pollen size of *Canna* samples is from 50 to 110 micron, fertility varies within a broad range – from 0 to 93 % [30, 31]. Mature pollen grains are two-celled; spermiogenesis takes place in a pollen tube [32, 33]. About 20–25 thousand of pollen grains are formed in an anther of *Canna* [27].

3. The aim and objectives of the study

The aim of this work was the need to create new varieties of *Canna*, which will be maximally adapted to the different ecological and climatic conditions of Kryvyi Rih regions, as well as the selection of parental pairs by determining the pollen fertility index of introduced species and varieties of different groups of *Canna*. The growing need in homegrown varieties of ornamental plants is based on economical practicability of the development of own selection database too.

To accomplish the aim, the following tasks have been set:

1. To determine the pollen quality index (fertility index) of introduced species and varieties of *Canna*.

2. To identify the promising parental forms by the fertility index.

3. To reveal the most effective combinations of parental forms of introduced species and varieties of *Canna*.

4. To create the selection fund of samples of *Canna,* which can be suitable for growing in the steppe zone of Ukraine.

4. Materials and Methods

The research was done on 7 species and 28 varieties of *Canna* of this collectors' fund. Pollen was chosen in the finishing phase of budding for the cytologic analysis and fertility was being defined by the iodine reaction of starch, according to the approved method [34, 35].

An attempt to preliminarily evaluate the feasibility of using varieties and species of *Canna* for artificial hybridization was undertaken. The ratio of fertile and sterile pollen is used ("fertility index" hereinafter, the designation F1is proposed). Hybridization was brought into action using known techniques for *Canna* [36]. In this case, castration of the flower was carried out in the phase of the ripe bud [14, 37]. With the opening of *Canna* flower, the negative impact of exogenous factors on the pollen state increases. Therefore, the pollination of flowers was carried out in the morning in the sunny weather (from 6:00 to 10:00) to avoid active drying of pollen [38].

We found that in conditions of the Kryvyi Rih region these works are the most effective recommended to do from the third decade of August to the second decade of September in the period of mass *Canna* budding [37]. The statistical processing of received results was conducted according to the common method (t-Student et. all) [39] using modern software (M. excel, Past).

5. Results and discussion

The fertility of pollen of *Canna* was studied in different phases of the development of a plant among varieties and species, which is in condition of the Kryvyi Rih region, when the vegetation finishes by fruit forming. The analysis of the pollen fertility at the different development phases of a flower showed that introduced varieties and species of *Canna* has the different potential of the reproductive function in condition of the Kryvorizhzhia. Only a small part of the studied varieties formed up to 50 % of viable pollen in the closed bud. These data are almost in agreement with the results of the evaluation of male generative structures in the cultivation of *Canna* in more favorable conditions [14, 31]. At the same time, we observed a clear tendency of loss of the pollen's vital potential, when comparing it in a closed bud and in an open flower [37]. The fertility of pollen of *Canna* species and varieties can vary greatly depending on the growing conditions or the characteristics of the growing season too [40, 41]. In particular, we have found that in the conditions of the Kryvyi Rih region, the highest fertility of *Canna* pollen was before the plant flowering. It is confirmed, that pollen of *Canna* has higher fertility in the final period of budding – before opening of a flower, that's why it was being selected for pollinating of maternal forms on hybridization in this phase [13, 38].

The average size of fertile pollen grains in species of *Canna* in the Kryvorizhzhia varies from 66–69 μm (in bud), 61–66 μm (in flower); in varieties of the Crozy group varies from 77–99 μm (in bud), 72–92 μm (in flower); in varieties of the Italian group varies from 86–105 μm (in bud), 73–94 μm (in flower) (Fig.1) [34].

Average pollen sizes of *Canna* specimens from the Nikita Botanical Gardens' collection range from 50 to 110 microns, fertility ranges widely from 0 to 93 % [14, 31]. Earlier, we established a significant loss of biometric and vital parameters of pollen of the variety Otblesk Zakata and the variety Podarok Kryma at the introduction from the Crimea to the Kryvorizhzhia. The average sizes of ripe pollen grains of the variety Otblesk Zakata decreased by 3.6 %, of the variety Podarok Kryma – by 8.2–10.3 %, and the fertility decreased by an average of 24 % [6].

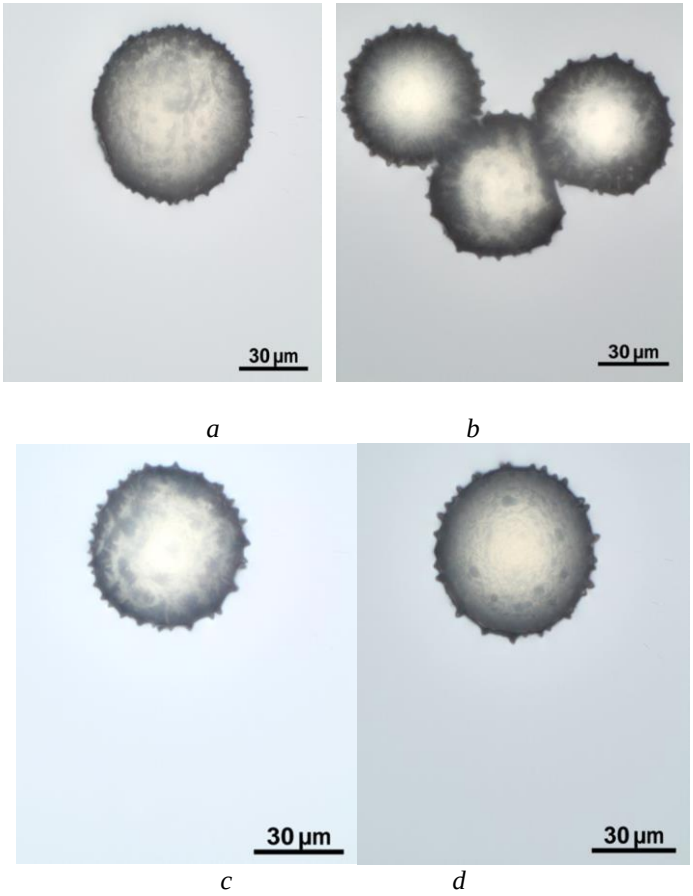

Fig. 1. The pollen of *Canna*: *a* – *C. hybrida* variety of the Crozy group; *b* – *C. hybrida* variety of the Italian group; *c* – *C. indica*; d – *C. iridiflora*

The maximum of pollen fertility in inspected species of *Canna* was 70–72 % during cultivation in the collection of KBG (Fig. 2). This is a much lower figure than the status of the male generative structures of *Canna*, when grown on the southern coast of Crimea. For example, in *C. indica* up to 85.42 % of morphologically normal pollen grains are formed there [14]. Only a third of the pollen formed was fertile in the final period of budding in the vast majority of introduced species of *Canna* in the Kryvorizhzhia (Fig. 3, 4).

It is noted, that in the Italian group (orchid flowering) *Cannas* varieties with the potentially high living index of pollen (FI <0.5) are missing (Fig. 3). Certain prospectivity can be predicted only with using parental forms in the

hybridization scheme for varieties Kapitan Iarosh, Ludmila and Suevia, FI of which is 0.60–0.65 relevantly.

The total number of investigational pollen grains was from 426 to 682 pieces.

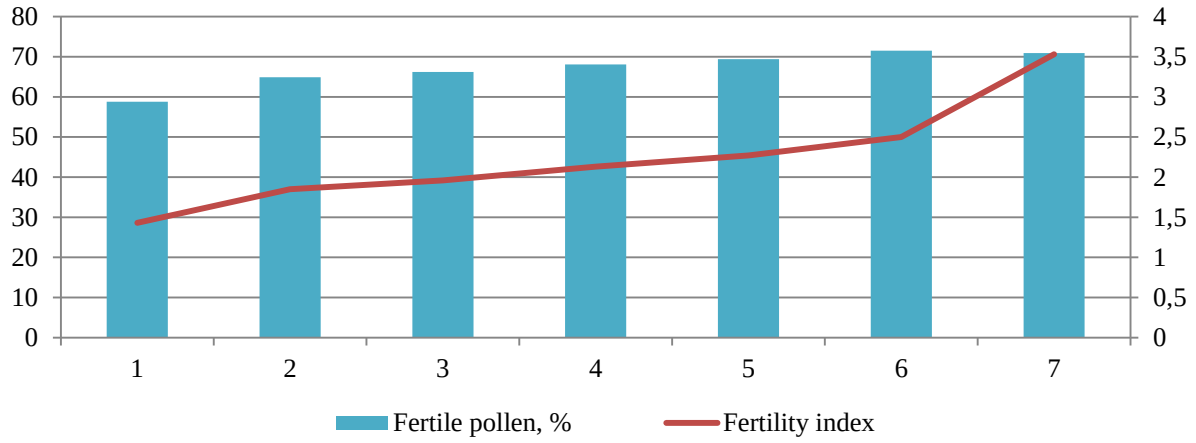

Fig. 2. Reference of fertile pollen of the inspected species of *Canna*: 1 – *C. tuerckheimii*; 2 – *C. indica var. warscewiczii*; 3 – *C. iridiflora*; 4 – *C. flacida*; 5 – *C. indica var. edulis*; 6 – *C. indica var. coccinea*; 7 – *C. indica*

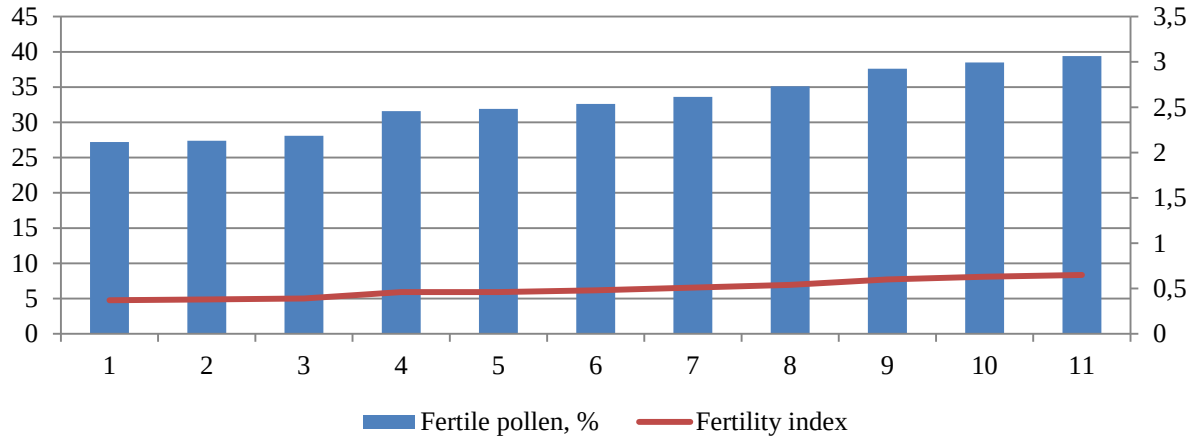

Fig. 3. Reference of fertile pollen of the variety of the Italian group of *Canna*:1 – Andenken an Wilgelm Phfitzer; 2 – Rosenkranzen; 3 – Orang Perfection; 4 – Fauervogel; 5 – Liberty; 6 – Prestizh; 7 – Mister Crozy; 8 – Konig Humbert; 9 – Kapitan Yarosh; 10 – Suevia;
11 – Lyudmila. The total number of investigational pollen grains is from 413 to 921 pieces

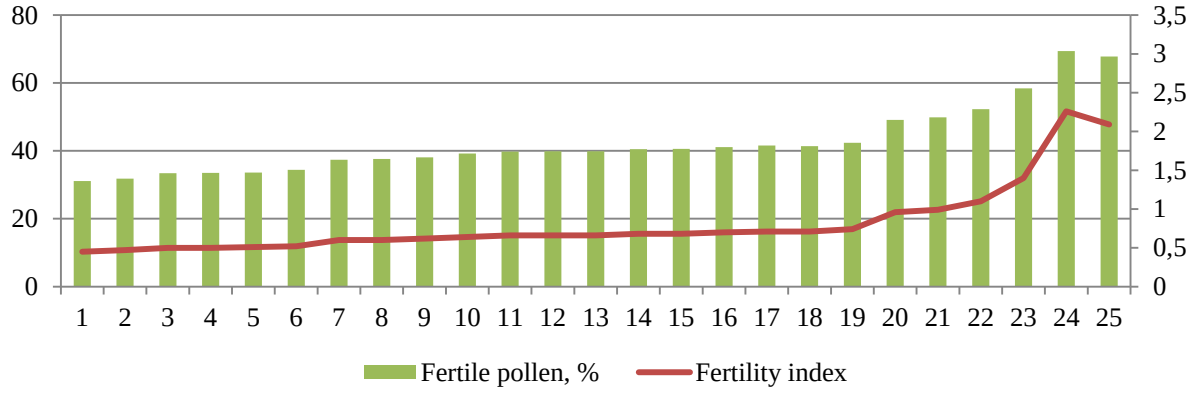

Fig. 4. Reference of fertile pollen of the variety of the Crozy group of *Canna*: 1 –Krymskie Zori; 2 – Klara Kurtik; 3 – Louis Cayeux; 4 – The President; 5 – Clara Buisson; 6 – Richard Wallis; 7 – Luis Cottin; 8 – America; 9 – Orang Punch; 10 –Solnechnaia Krasavica; 11 – Vostok-2; 12 – Hameleon; 13 – Futurity Yellow; 14 – Dar Vostoka; 15 – Ai-Petri; 16 – Vesielye Notki; 17 –Podarok Kryma; 18 – Capter; 19 – Apricot Dream; 20 – Salyut Pobedy; 21 – Madam Angel; 22 – Shedevr; 23 –Otblesk Zakata; 24 – Nadezhda; 25 – Maestro

The total number of investigational pollen grains is from 425 to 752 pieces.

Among species of the Crozy group variety for the uttermost result it is better to use varieties with the distinct predominance of fertile pollen in the bud (from FI=0.7 to FI= 1.4) as parental forms for artificial cross (See fig. 4). Using varieties with FI< 0.5 as parental forms is of little promise (Krymskie Zori, Klara Kurtik, Louis Cayeux and The President). Most varieties of this group have FI=0.7 and in particular they were widely involved in hybridization schemes.

It has been found, that in the population of studied pollen of *Canna* varieties the part of fertile pollen in a bud exceeds the quantity of sterile seeds, at least in 1.5–2 times, for *C. coccinea* at 2.5 and *C. indica* in 3.5 times. It means, at first, that life opportunities of *Canna* pollen are significantly lower than in other varieties, and, at second, it has a distinction as to a variety belonging to a certain variety group.

In our work the usage of varieties, FI of which ≅1.0 (or FI >1.0) were the most effective in the hybridization. So, pollination took place quite successfully with the following formation of hybrid seeds in combinations with the usage of varieties Madam Angel, Shedevr, Orange Beauty, Otblesk Zakata.

Having the aim of creation of the selection fund of *Canna* with wide varieties of samples of the \ great ornamental use, hybridization schemes were made in several ways:

1) in different variations of varieties' combinations from different variety groups;

2) usage of a variety as a maternal or paternal form in artificial crossing with introduced species.

As a result of some stages in hybridization work, 79 selection schemes were researched, from which 63 pairs of intervarietal crossing, 13 variants with the usage of the native kind as a mother form, 3 variants with the usage of the native kind as a father form. The whole number of artificially pollinated buds which were analyzed in different crossing combinations is 3097 (Table 1).

Table 1

The most promising combinations of artificial hybridization

The combinations of parental pairs	Total pollinated buds, pcs	Pollinated buds on 1 plant, mean ± SD	Total flowers after pollination, %	The flower is pollinated on 1 plant, mean ± SD	Fetal-fruitplant-ingseeds, %
The crossing among varieties ♀ – variety of Italian group ×♂ variety of the Crozy group					
Liberty×America	34	6.8±0.37	14.71	1.2±0.25	60.00
♀ and ♂ – variety of the Crozy group					
Ai-Petri×Otblesk Zakata	72	14.4 ±0.55	30.60	4.4±0.20	27.30
Ai-Petri×Podarok Kryma	34	6.8±0.58	23.53	1.6±0.40	62.50
Hameleon×Krymskie Zori	64	12.8±0.47	28.12	3.6±0.37	22.22
Podarok Kryma×Otblesk Zakata	36	7.2±0.73	25.00	1.8±0.37	88.89
Madam Angel × Krymskie Zori	38	7.6±0.24	26.32	2.0±0.55	50.00
Capter×Shedevr	37	7.4±0.51	16.22	1.5±0.29	16.67
Vesielye Notki×Ai-Petri	35	7.0±0.45	25.71	1.8±0.58	55.56
MadamAngel×SolnechnaiaKrasavica	36	7.2±0.37	27.78	2.0±0.32	80.00
Podarok Kryma× Solnechnaia Krasavica	34	6.8±0.58	20.59	1.4±0.40	42.80
♀- variety of the Crozy group ×♂ variety of the Italian group					
Krymskie Zori×Prestizh	74	14.8±0.54	35.13	5.2±0.60	7.70
Maestro × Lyudmila	34	6.8±0.58	20.59	1.4±0.24	28.57
Shedevr×Prestizh	38	7.6±0.51	13.16	1.2±0.25	20.00
Shedevr×Orang Beauty	37	7.4±0.51	21.62	1.6±0.40	62.50
The crossing among introduced species and varieties ♀ – introduced species ♂ – variety of the Crozy group					
C. indica var. coccinea Mill. ×Ai-Petri	40	8.0±0.71	25.00	2.0±0.45	30.00
C. indica var. coccinea Mill.×The President	38	7.6±0.51	39.47	3.0±0.77	60.00
C. flacida Salisd. ×Richard Wallis	37	7.4±0.40	45.95	3.4±0.40	5.88
♀ – introduced species ♂ – variety of the Italian group					
C. indica var.coccinea Mill. ×Fauervogel	32	6.4±0.40	46.88	3.0±0.32	73.30
C. *indica* L.×Lyudmila	35	7.0±0.71	40.00	2.8±0.49	7.14
C. indica var. warscewiczii Nob.Tanaka × Prestizh	34	6.8±0.58	20.00	1.2±0.25	20.00

It has been noted, that the most promising (30.6 %) combinations were received by crossing among varieties of the Crozy group. From 15 parental pairs, in which variety of the Italian group *Canna* was a mother form and variety of the Crozy group was a father form, only one variant (♀Liberty×♂America) gave hybrid

seeds. Crossing variants among Italian group varieties turned out to be absolutely unfertile.

In general, only 22.2 % variants among variety combinations gave a successful seed formation and 46.2 % – variants with the usage of species as a mother form. The combinations of parental pairs, in which mother was a variety and father was introduced species, appeared to be unfertile.

In crossing among varieties only 2 seeds are formed in a formed fruit bud, whereas species have about 6–8 seeds. In our researches the highest results as to the seed formation (from potentially possible) have been stated for variants of intervarietal crossing with the usage of the Crozy varieties group as parental forms (in average – 49.5 %). With the usage of varieties of this group as a maternal form (in average – 29.7 %) the seed formation was rather successful too.

In fact, only 20 parental forms from all researched combinations of crossing formed fruits (which amounts 25.3 %) (Table 1). The most successful variants of intervarietal hybridization turned out to be the following:

♀Ai-Petri × ♂Otblesk Zakata,
♀Ai-Petri × ♂Podarok Kryma,
♀Podarok Kryma × ♂Otblesk Zakata,
♀Madam Angel × ♂Solnechnaia Krasavica,
♀Madam Angel × ♂Krymskie Zori,
♀Vesielye Notki × ♂Ai-Petri,
♀Shedevr × ♂Orange Beauty,
♀Liberty × ♂America.

In this case in average one seed was formed in a fruit bud, which is a variety characteristic of *Canna*.

The further study of phenotypic characters of received hybrid flowers with species usage in selection work showed the absence of forms of the ornamental use in obtained breeding. It is probably connected with the domination of characters of the introduced species, used in crossings. A larger complex of valuable ornamental characteristics was observed in hybrid forms, obtained in variants among variety combinations; especially, where the parental forms used varieties of the Crozy group. According to the results evaluation as to qualitative and quantitative characteristics from pollination of the hybrid breed, 2 selection forms were pointed according to the complex of characteristics of the ornamental use (Fig. 5).

A

a *b* *c*

B

a *b* *c*

Fig. 5. The parental pairs and hybrid forms obtained. A: *a* – ♀ Podarok Kryma; *b* – ♂ Solnechnaia Krasavica; *c* – The hybrid form 1-1-2011. B: *a* – ♀ Madam Angel; *b* – ♂ Solnechnaia Krasavica; *c* – The hybrid form 9-7-2011

The hybrid form 1-1-2011 was marked in the combinations of crossings ♀Podarok Kryma× ×♂Solnechnaia Krasavica. The plant has a bright green leaf color as in the parent variety. The color of staminodias of the resulting hybrid is a distinctive feature. There is a combination of two colors, inherited from the parent varieties with the dominance of carmerinusruber color (45 C) and slavjanskiisolaris color (3 A) (with RHS Colour Chart) [42]. The hybrid form 9-7-2011 have been pointed in the combinations of crossings ♀Madam Angel × ♂Solnechnaia Krasavica. The plant has leaves of a gray-green color and bright yellow flowers as in the par-

ent form. Orange dotted all over the flower is a decorative addition (punctuated). The hybrid plant F_1 inherited the shape of the flower as in the mother variety, but the size was smaller. This is especially valuable in accordance with the requirements of modern floriculture for created varieties of *Canna*.

6. Conclusions

1. According to the index of pollen quality (FI), in the final period of bud formation the prospective introduced species *Canna* were marked for further usage as parental forms in hybridization schemes.

2. In varieties that had an FI of approximately 1.0 (or more), the results of artificial hybridization were higher. The average FI values for the introduced species were 2.3; for the varieties of the Crozy group – 1.1. The prospectivity of using Italian group varieties as parental forms proved to be very low, determined by the FI that did not exceed 0.65.

3. On the basis of performed artificial hybridization the most effective combinations of crossings with the efficiency of seed formation above 50 % were found as promising introduced *Canna* varieties for the steppe zone and industrial region of Ukraine. It should be noted, that the most effective combinations were obtained when crossing among the varieties of the Crozy group.

4. We matched parental pairs for artificial hybridization of *Canna* with the aim of genetic and selective enrichment of its varieties and getting samples, which can be suitable for growing in the steppe zone of Ukraine, especially in the ecologically destabilizing region. From the received fund of hybrid *Canna* flowers 2 selection forms have been pointed out as to the complex of flowers with a characteristic of the great ornamental use and have been introduced to the collection fund for their further implementation in landscape gardening.

Conflict of interest

There is no conflict of interest

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